Figure 2 Association between baseline HR and outcomes in HFrEF and HFpEF. Adjusted hazard ratio for all-cause death, CV death, HF death, HF admission, and non-CV death in HFrEF and HFpEF.

Table 2 Unadjusted and adjusted hazard ratios of β-blocker for all-cause death, CV death, HF death, and HF admission in HFrEF and HFpEF

	Unadjusted				Adjusted[a]			
	HFrEF		HFpEF		HFrEF		HFpEF	
	Hazard ratio (95% CI)	P-value	Hazard ratio (95% CI)	P-value	Hazard ratio (95% CI)	P-value	Hazard ratio (95% CI)	P-value
All-cause death	0.70 (0.50–0.99)	0.042	0.81 (0.59–1.10)	0.174	0.71 (0.49–1.03)	0.075	1.11 (0.79–1.54)	0.553
CV death	0.68 (0.43–1.06)	0.088	0.79 (0.49–1.27)	0.326	0.68 (0.42–1.11)	0.123	0.96 (0.58–1.59)	0.874
HF death	0.51 (0.28–0.94)	0.030	0.51 (0.23–1.13)	0.096	0.49 (0.25–0.96)	0.038	0.64 (0.26–1.55)	0.321
HF admission	1.16 (0.84–1.63)	0.351	0.98 (0.68–1.41)	0.926	1.05 (0.74–1.49)	0.797	0.97 (0.66–1.43)	0.887

[a]Adjusted by age, sex, BMI, systolic blood pressure, LVEF, LVDd, Hb, eGFR, RASI, and HR catgories.
CV, cardiovascular; HF, heart failure.

in HFrEF. The results demonstrated that the impact of elevated baseline HR on CV mortality was notable in the HFpEF group compared with the HFrEF group, particularly on HF mortality.

Elevated baseline HR and all-cause mortality in HF

The present study demonstrated the impacts of HR status on all-cause mortality in both HFrEF and HFpEF patients, where the increased risk of all-cause deaths in patients with higher HR was noted even after adjustment for patient background, medication, and possible other confounders for mortality and morbidity. This relationship between elevated baseline HR and increased mortality appears to be reasonable in the clinical setting, because elevated HR could be a reflection of neurohumoral activation of the sympathetic nervous system, an excessive compensation for reduced cardiac output and myocardial ischaemia. However, it is still controversial whether elevated baseline HR is associated with increased all-cause mortality in HFpEF as in HFrEF. For example, in the subanalysis of the CHARM programmes, the correlation of baseline HR and risks for all-cause death was noted in both the HFrEF and HFpEF groups,[11] whereas the subanalysis of the DIG study revealed that elevated HR was associated with all-cause death in HFrEF but not in HFpEF patients.[13] Thus, our results regarding prognostic impacts of elevated HR on all-cause mortality are consistent with those of the CHARM programmes, but not with those of the DIG study, providing additional evidence for the relationship between baseline HR and clinical outcomes in a large cohort of patients receiving contemporary management for Stage C/D HF in the real-world setting.

Table 3 Split point of HR for outcomes in overall patients, HFrEF and HFpEF

		First split point of HR	Hazard ratio of higher HR group[a]	95% CI	P-value
CV death	All	63.5 bpm	1.85 (≥ 64 bpm)	1.26–2.73	0.002
	HFrEF	69.5 bpm	1.60 (≥ 67 bpm)	1.00–2.55	0.051
	HFpEF	63.5 bpm	2.04 (≥ 64 bpm)	1.17–3.53	0.012
Non-CV death	All	71.5 bpm	1.68 (≥ 72 bpm)	1.19–2.38	0.004
	HFrEF	71.5 bpm	1.34 (≥ 72 bpm)	1.22–4.50	0.011
	HFpEF	71.5 bpm	1.45 (≥ 72 bpm)	0.95–2.22	0.082

[a]Unadjusted hazard ratios of patients with HR more than optimal split point indicated by CART analysis (higher HR group) over those with HR not more than indicated (lower HR group). The minimum HRs of the higher HR group are shown in parentheses next to the hazard ratios.

Different impact of baseline HR between HFrEF and HFpEF

Bui et al. demonstrated that HFpEF was associated with a higher risk of in-hospital mortality with increasing admission HR compared with HFrEF among patients hospitalized for HF, suggesting that higher HR might have imparted increased in-hospital mortality in HFpEF patients.[20] As for the impacts of elevated baseline HR on long-term CV mortality, the present study may provide the first evidence that such impacts on CV death, particularly on HF death, are rather significant in HFpEF compared with HFrEF (Figure 2). The relationship between elevated HR and increased CV mortality in HFpEF appears reasonable, since HFpEF is generally complicated by diastolic dysfunction and thus could be further worsened by shortening of the diastolic period according to an increase in HR.[21] In the present study, there was no association between HR and hospitalization for HF in HFrEF or HFpEF (Figure 2). In addition, the present study may provide the first evidence for the association between baseline HR and non-CV death in HFrEF patients, following an association between HR and non-CV mortality being observed in the general population.[22–24] Although the precise mechanisms remain to be elucidated, low physical activity, elevated adrenergic activity and smoking might be possible explanations for the association between elevated HR and increased non-CV mortality.[22–24]

Cut-off value of HR for CV death in HFrEF and HFpEF

In order to determine the cut-off point for HR to partition Stage C/D patients according to the mortality rates, we performed CART analysis, demonstrating that 63.5, 69.5, and 63.5 bpm could be the primary splitting points for CV death among the overall, HFrEF, and HFpEF patients, respectively (Table 3). The univariate Cox regression analysis revealed that HFpEF patients with HR ≥ 63.5 bpm had an increased risk for CV death with a statistical significance (hazard ratio 2.04, P = 0.012 for patients with HR ≥ 64 bpm), and HFrEF patients with HR ≥ 69.5 bpm with a tendency (hazard ratio 1.60, P = 0.051 for patients with HR ≥67 bpm). These results may suggest that the therapeutic range of HR to reduce CV mortality could be lower in HFpEF compared with HFrEF patients (63.5 vs.

69.5 bpm). This was likely because a longer duration of the diastolic period is necessary to reduce CV mortality in patients with diastolic dysfunction compared with systolic dysfunction. In this context, HR reduction therapy could be an option to reduce CV mortality in HFpEF patients. Indeed, it has been reported that selective HR reduction by ivabradin improves vascular stiffness and left ventricular systolic and diastolic function in mice.[25] A subanalysis of the SHIFT trial, which enrolled patients with HF and EF < 35%, revealed that the prognostic impact of HR reduction by ivabradine was greater in patients who had baseline HR ≥ 75 and had achieved < 60 bpm or heart rate reductions > 10 bpm.[26] Although the cut-off point of HR to discern CV mortality may vary according to the baseline ejection fraction, further reduction of HR with ivabradine could be effective in patients with HFpEF. However, further investigations are required to elucidate whether HR reduction is effective in the management of HFpEF patients in real-world practice.

β-Blocker therapy in HFpEF

It is widely accepted that β-blocker therapy improves LVEF and reduces mortality in HFrEF patients through inhibition of sympathetic nervous activity and reduction in HR and oxygen consumption.[27,28] The present study suggested different prognostic impacts of β-blockers between HFrEF and HFpEF, as β-blocker therapy was associated with decreased HF mortality in patients with HFrEF but not in those with HFpEF. β-Blockers could theoretically be beneficial in patients with HFpEF because shortening of the diastolic period could exacerbate diastolic dysfunction, a common feature of the disorder.[21] However, it was previously reported that β-blockers may not be so useful in HFpEF patients,[29] a consistent finding of the present study. However, there remains a possibility that standard doses of β-blockers (for Japanese patients) in the present study was not sufficient to reduce CV mortality for HFpEF patients. In fact, Yamamoto et al. recently reported that a higher dose of carvedilol was associated with lower incidence of a composite of cardiovascular death and unplanned hospitalization for any cardiovascular cause in patients with HFpEF in the Japanese population.[30] Thus, further studies are warranted to examine whether higher doses of β-blockers could improve the mortality of HFpEF patients.

Study limitations

Several limitations should be mentioned for the present study. First, the number of HFrEF patients was smaller than that of HFpEF patients, and therefore the power might not be enough to detect a statistical significance in HFrEF patients; thus, interpretation should be made with caution. Second, the CHART-2 Study is a prospective, observational study that reflects the real-world practice of HF, as consecutive HF patients were enrolled with a minimal selection bias; however, we have to consider influences on the results by unknown confounders. Third, in the present study, we only used the data at the entry and did not take into consideration the possible changes in LVEF, HR, episodes of arrhythmia, particularly those of atrial fibrillation, medication, and other covariates during the follow-up period. In addition, no data were available for β-blocker therapy, such as timing of initiation, daily doses, adherence, discontinuation, and reasons for the presence or absence of prescription. Thus, it was difficult to elucidate the prognostic impact of β-blocker therapy in the present study. Fourth, in the present study, according to European Society of Cardiology guidelines,[15] we chose the cut-off value of LVEF 50% to define HFpEF. However, caution is needed in interpreting the present results when comparing other cohorts with different cut-off values to discriminate between HFrEF and HFpEF, such as 35% or 40%.[8,10] Finally, all subjects in the CHART-2 Study were Japanese people, which may limit generalization of the present results to patients in other countries.

Conclusions

We demonstrated the different impacts of elevated baseline HR on CV and non-CV mortality between HFrEF and HFpEF in the CHART-2 Study. Although the influence of elevated baseline HR on all-cause mortality was comparable, elevated HR was significantly associated with CV death in HFpEF, but insignificantly in HFrEF, particularly for HF death. Further studies are needed to elucidate the relationship between elevated baseline HR and mortality in order to improve the survival of HF patients.

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Supplementary Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Baseline characteristics of tertiles defined by baseline heart rate of the two groups
Table S2. Actual number of event for tertiles in HFrEF and HFpEF
Table S3. Baseline characteristics across four groups defined by LVEF and β-blocker
Appendix S1. Impact of elevated heart rate on clinical outcomes in patients with heart failure with reduced and preserved ejection fraction: a report from the CHART-2 study

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Association between Myocardial Triglyceride Content and Cardiac Function in Healthy Subjects and Endurance Athletes

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Abstract

Ectopic fat accumulation plays important roles in various metabolic disorders and cardiovascular diseases. Recent studies reported that myocardial triglyceride (TG) content measured by proton magnetic resonance spectroscopy (^{1}H-MRS) is associated with aging, diabetes mellitus, and cardiac dysfunction. However, myocardial TG content in athletes has not yet been investigated. We performed ^{1}H-MRS and cardiac magnetic resonance imaging in 10 male endurance athletes and 15 healthy male controls. Serum markers and other clinical parameters including arterial stiffness were measured. Cardiopulmonary exercise testing was also performed. There were no significant differences in clinical characteristics including age, anthropometric parameters, blood test results, or arterial stiffness between the two groups. Peak oxygen uptakes, end–diastolic volume (EDV), end–systolic volume (ESV), left ventricular (LV) mass, peak ejection rates and peak filling rates were significantly higher in the athlete group than in the control group (all P<0.02). Myocardial TG content was significantly lower in the athlete group than in the control group (0.60±0.20 vs. 0.89±0.41%, P<0.05). Myocardial TG content was negatively correlated with EDV (r=−0.47), ESV (r=−0.64), LV mass (r=−0.44), and epicardial fat volume (r=0.47) (all P<0.05). In conclusion, lower levels of myocardial TG content were observed in endurance athletes and were associated with morphological changes related to physiological LV alteration in athletes, suggesting that metabolic imaging for measurement of myocardial TG content by ^{1}H-MRS may be a useful technique for noninvasively assessing the "athlete's heart".

Introduction

Ectopic fat accumulation is associated with various metabolic disorders and cardiovascular diseases [1–3]. Previous animal studies have shown that myocardial triglyceride (TG) accumulation triggers pathological changes, including myocardial apoptosis and ventricular systolic dysfunction [4,5]. However, the assessment of myocardial TG content is hampered by the difficulty of obtaining myocardial tissues in a clinical setting.

Recent studies have demonstrated that proton magnetic resonance spectroscopy (^{1}H-MRS) enables the noninvasive monitoring of TG accumulation in human myocardial tissue. Indeed, myocardial TG content, as measured by ^{1}H-MRS, has been associated with aging [6], diabetes mellitus [7], myocardial systolic dysfunction [4,8,9], and diastolic dysfunction [6,10]. In addition, caloric restriction induced a dose-dependent increase in myocardial TG content [11], whereas endurance training reduced myocardial TG content [12]. However, myocardial TG content in endurance athletes has not yet been investigated.

The purpose of this study is to evaluate the associations between myocardial TG content, cardiac morphology and left ventricular (LV) function assessed by ^{1}H-MRS and magnetic resonance imaging (MRI) in healthy subjects and endurance athletes.

Methods

Subjects

Fifteen healthy male subjects and 10 male endurance athletes were recruited by advertisements in a local area. All subjects were non-obese, aged 20–40 years, and without acute or chronic disease. Subjects receiving medical treatment, current smokers, and those with abnormal laboratory parameters were excluded. We defined an endurance athlete as a person who performed endurance training for more than 5 days a week, and was affiliated with a specific athletic association to participate in competitive sports such as cycling, track, or swimming. The international physical activity questionnaire (IPAQ) was used to assess each subject's activity level [13]. All protocols were approved by the ethical committee of the Juntendo University, and all participants

provided written informed consent before their participation in this study according to the guidelines established in the Declaration of Helsinki.

Measurements of Body Composition

Skeletal muscle mass and body fat weight were measured after overnight fasting by multi-frequency bioelectrical impedance analysis using eight tactile electrodes (MF-BIA8; In-Body 720, Biospace, Korea) [14] after overnight fasting. The subject stood on the footplate with barefoot and held the electrodes in both hands. This process takes 2 min, and measurement requires no specific skills. The apparatus then automatically displays measurements of fat-free mass, fat mass, and percentage body fat.

Blood Measurements

Standard laboratory tests including blood cell counts, fasting plasma glucose, lipids, creatinine, free fatty acid, and N-terminal pro-brain natriuretic peptide (NT-proBNP) were performed immediately before MRS after overnight fasting. Serum lipid profiles were measured using specific assays for total cholesterol (Symex Co, Kobe, Japan), triglyceride (Sekisui Medical, Tokyo, Japan), and high-density lipoprotein cholsterol (Sekisui Medical, Tokyo, Japan) by BioMajesty JCA-BM8060 analyzer (Japan Electron Optics Laboratory Ltd, Tokyo, Japan). Serum low-density lipoprotein cholesterol levels were calculated using the Friedewald's formula. Serum insulin was measured by chemiluminescent enzyme immunoassay using the Lumipulse presto II analyzer (Fujirebio Inc, Tokyo, Japan). A homeostasis model assessment index (HOMA-IR) was calculated to estimate insulin resistance from fasting insulin and glucose concentrations: insulin (μU/ml)$\times$glucose (mmol/l)/22.5. Free fatty acid (FFA) was measured a standard enzymatic assay (Eiken chemical Co. Ltd, Tokyo, Japan) by BioMajesty JCA-BM2250 analyzer (Japan Electron Optics Laboratory Ltd, Tokyo, Japan). Serum NT-proBNP was determined using an electrostatic controlled linear inchworm actuator on Hitachi modular analytics (HITACHI Hi-Technologies Co. Ltd. Tokyo, Japan). HbA1c concentrations were measured in whole blood samples using latex-enhanced immuno-assay (Fujirebio Co. Ltd. Tokyo, Japan).

MRI and MRS

All cardiac MRI and ^{1}H-MRS studies were performed using a MAGNETOM Avanto 1.5-Tesla MRI system (Siemens Medical Solution, Erlangen, Germany) with subjects resting in the supine position. To minimize the influence of breathing, a towel was strapped around the subject's upper abdomen. Dynamic cine images were used to determine LV mass, and LV functional parameters. Image analysis was performed using special evaluation software (Argus; Siemens Medical Systems, Erlangen, Germany) [15,16] on a separate work station. Endocardial and epicardial LV borders were traced manually at end-diastole and end-systole from short-axis cine images. End-diastolic volume (EDV), end-systolic volume (ESV), stroke volume, and ejection fraction were calculated by Simpson's method. In addition, the peak LV ejection and filling rates were automatically derived on the basis of LV volume-time curves. The area of epicardial fat was traced on consecutive end diastolic short axis images, beginning with the most basal slice at the level of the mitral valve, and moving apically through the stack until the most inferior margin of adipose tissue, as reported previously [17].

After the cine MRI imaging, myocardial TG content was determined by ^{1}H-MRS. A volume of interest (VOI = 2.0 cm^3 − 10×10×20 mm) was selected within the ventricular septum from cine dynamic cine-mode images of the heart (Figure 1). We adjusted the VOI size to the anatomy of the ventricular septum. The spectrum of water and lipid was acquired by point-resolved spectroscopy (PRESS) method using an echo time (TE) of 30 ms, and repetition time (TR) of at least 4,000 ms, myocardial TG signals were acquired at 1.4 ppm from spectra with water suppression, and water signals were acquired at 4.7 ppm from spectra without water suppression (Figure 1). Areas under the curves for water and lipid peaks were quantified using standard line-fitting procedures (Siemens Syngo Spectroscopy). Myocardial TG level was expressed as a ratio of lipid to water (%). Thus, ^{1}H-MRS evaluation of myocardial TG content was performed essentially as has been previously validated [18–21].

Measurement of Cardiopulmonary Fitness

All subjects underwent an incremental cycling test (Corival 400, Lobe B.V., Groningen, Netherlands) using an expiratory gas analyzer (Vmax-295, sensorMedics Co., Yorba Linda, CA, USA) to measure anabolic threshold (AT) and maximal oxygen consumption (VO$_{2max}$). After a 3-min rest period, a warm-up was performed for 3 minutes at 40 W, followed by ramp loading (15–30 W/min) until the subjective exhaustion, as described previously [22]. According to the ATS/ACCP guidelines, AT was determined by V-slope method. In cases when AT was not identified on the V-slope, we used the point at which V$_E$/VO$_2$ starts to increase while V$_E$/VCO$_2$ remains constant [23].

Evaluation of Atherosclerotic Parameters

The cardio ankle vascular index (CAVI) was measured as atherosclerotic parameters. CAVI was automatically calculated by VaSera VS-1500AN (Fukuda Denshi Co. Ltd., Tokyo, Japan) [24,25].

Statistical Analyses

Values are expressed as mean $\pm$ standard deviation (SD). For variables that did not show a normal distribution, the data were transformed into natural logarithmic values before statistical analyses. Correlations were calculated using Pearson's correlation co-efficient. Unpaired Student's t-test was used to compare groups. All statistical analyses were performed with SPSS version 20 (SPSS, Inc). A P value of less than 0.05 was considered significant.

Results

The clinical characteristics of study subjects are summarized in Table 1. There were no significant differences, in age, body composition, lipids, glucose, insulin levels, or NT-proBNP between the two groups. The levels of AT (29.2$\pm$6.6 ml/kg/min vs. 19.0$\pm$5.2 ml/kg/min, P = 0.0002), VO$_{2max}$ (52.3$\pm$6.2 ml/kg/min vs. 43.2$\pm$8.0 ml/kg/min, P = 0.0057) and international physical activity questionnaire (IPAQ) score (2318$\pm$1605 vs. 5310$\pm$2869, P = 0.0048) were significantly higher in the athlete groups than in the control group.

MRI and MRS variables are shown in Table 2. The values of EDV (182$\pm$24 ml vs. 153$\pm$16 ml, P = 0.0011), ESV (96$\pm$16 ml vs. 73$\pm$8 ml, P = 0.0002), and LV mass (139$\pm$16 g vs. 120$\pm$13 g, P = 0.0034), were significantly higher in the athlete group than in the control group. Peak ejection rate (777$\pm$230 ml/sec vs. 551$\pm$206 ml/sec, P = 0.019) and peak filling rate (839$\pm$250 ml/sec vs. 619$\pm$177 ml/sec, P = 0.018) were significantly higher in the athlete group than in the control group. None of the subjects had an abnormal peak ejection or filling rate. Myocardial TG content was significantly lower in the athlete group than in the control group (0.60$\pm$0.20% vs. 0.89$\pm$0.41%, P = 0.045) (Figure 2).
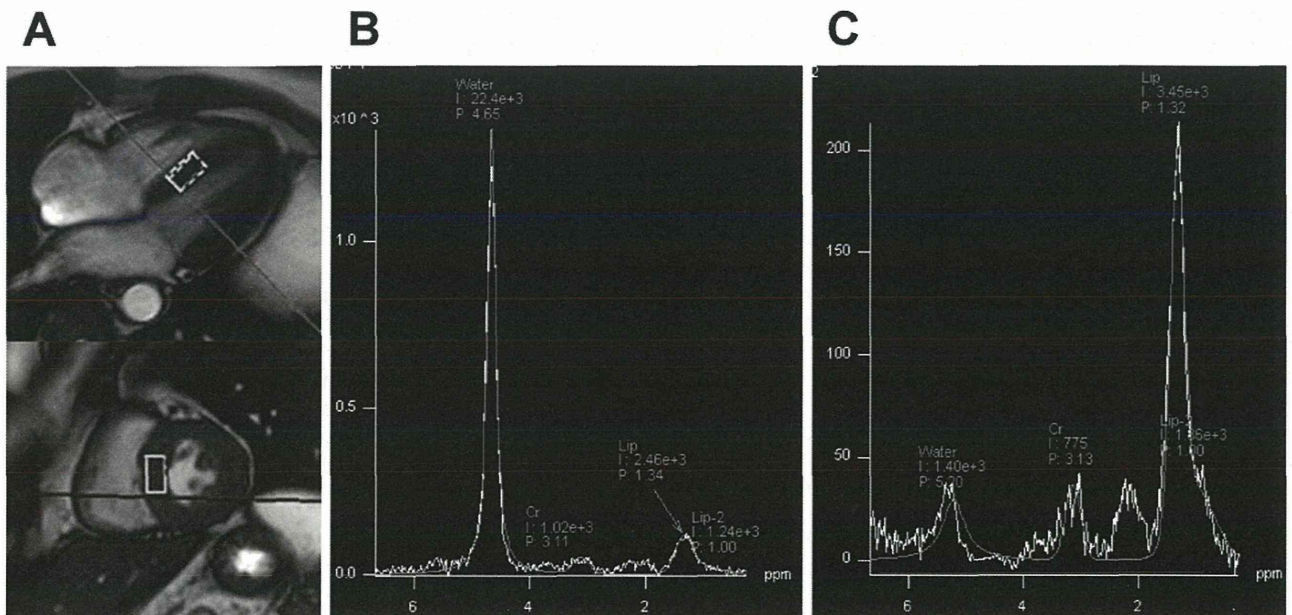
Figure 1. Representative results of H¹-MR spectra in a healthy subject. A: Myocardial voxel localization for H¹-MRS in 4-chamber and short axis views. B: H¹-MR spectra without water suppression. C: H¹-MR spectra without water suppression.
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Myocardial TG content was negatively correlated with EDV ($r = -0.47$, P = 0.018), ESV ($r = -0.64$, P = 0.001), LV mass volume ($r = -0.43$, P = 0.031), and epicardial fat volume ($r = 0.47$, P = 0.025) (Figure 3). Although a significant correlation between myocardial TG content and VO_{2max} was not found ($r = -0.15$, P = 0.46), epicardial fat volume was negatively correlated with EDV, a LV morphological parameter ($r = -0.44$, P = 0.022).

Discussion

The present study demonstrated that myocardial TG content was significantly lower in the endurance athlete group than in the control group and that myocardial TG content was significantly correlated with EDV, ESV, LV mass, and epicardial fat volume. This study is, to the best our knowledge, the first report to demonstrate an association between TG content and physiological LV alteration in endurance athletes.

Much attention has been focused on the associations between ectopic fat accumulation, various metabolic disorders and cardiovascular diseases [1,2]. It has been reported that the myocardial TG content is associated with metabolic disorders [7,26]. The positive correlation between myocardial TG content and LV mass has also been reported among the diabetic patients as well as in obese individuals with insulin resistance [4,8]. Animal studies have demonstrated that myocardial TG content was associated with not only cardiovascular risk factors, but also with lipotoxicity-induced heart failure and premature death [20,27]. In addition, increased myocardial TG content induced pathological LV hypertrophy, cardiac dysfunction, and non-ischemic dilated cardiomyopathy [28]. However, the present study showed negative correlations between myocardial TG and LV mass as well as LV function. Several studies suggested that mitochondrial dysfunction in the myocardium exists in patients with diabetes and insulin resistance [29]. In contrast, the functional capacity of mitochondria in athlete's heart was reported to be increased by

endurance training [30]. This difference in mitochondrial function may underlie the difference in myocardial TG content between the physiological modifications present in athlete's heart and the pathological changes that characterize the deteriorating heart in patients with diabetes and insulin resistance.

Previous studies reported the relationship between exercise and lipid content in skeletal muscle. High levels of intra-myocellular lipid (IMCL) were reported in the skeletal muscles of patients with diabetes mellitus [31] and elderly subjects [32]. On the other hand, it has also been reported that similar high levels of IMCL occur in skeletal muscles of athletes, despite the marked insulin sensitivity and the high oxidative capacity of these muscles, this is the so-called "athlete's paradox" [33]. Increases in IMCL content provide a substrate for energy metabolism during exercise [34]. A high availability of fatty acids is needed to augment TG resynthesis in skeletal muscle during and after exercise [34]. Diacylglycerol and/or ceramide, but not TG, may be directly associated with the development of insulin resistance [35,36]. In the present study, no "athlete's paradox" was observed in the subjects' cardiac muscles. Several potential reasons have been raised. One possibility is the difference in mitochondrial function with regard to fatty acid metabolism between skeletal muscle and cardiac muscle. Fatty acid metabolism may be more efficient in cardiac muscles, which has more abundant mitochondria than in skeletal muscles [37]. Another reason relates to the differences in regulation of fatty acid β-oxidation between the two types of muscle. To sustain contractile function in the heart requires a greater energy supply [38]. Therefore, the fatty acid β-oxidation system in cardiac muscle is very dynamic and sufficient to meet the energy demands of the heart. Alterations in lipoprotein lipase (LPL) synthesis as well as the activation, secretion, transportation, capillary luminal binding, and the degradation of fats in cardiac myocytes, contribute to myocardial fatty acid supply, uptake and fatty acid β-oxidation [38]. In addition, the heart muscle is reported to be less susceptible to developing insulin resistance than skeletal

Table 1. Clinical Characteristics.

	Control group (n = 15)	Athlete group (n = 10)	P value
Age, years	28.8±4.5	26.4±4.4	0.20
Body height, m	1.735±0.051	1.732±0.047	0.88
Body weight, kg	67.9±7.4	67.8±4.2	0.94
Body mass index, kg/m²	22.5±1.9	22.6±1.9	0.90
Skeletal muscle mass, kg	30.7±2.6	32.5±2.0	0.083
Body fat weight, kg	13.6±3.8	10.6±3.6	0.066
Percent of body fat, %	18.6±5.0	15.4±4.8	0.14
Neck circumference, cm	36.9±2.4	36.8±1.8	0.92
Waist circumference, cm	80.5±6.8	78.1±4.0	0.36
Total cholesterol, mg/dl	174.6±26.3	182.5±24.5	0.45
Triglyceride, mg/dl	74.6±27.0	61.1±15.8	0.16
LDL-cholesterol, mg/dl	104.2±26.4	111.1±29.0	0.53
HDL-cholesterol, mg/dl	55.7±11.3	59.2±12.7	0.47
Fasting free fatty acid, µEq/L	299.1±132.3	364.7±211.5	0.32
Fasting blood glucose, mg/dl	90.7±8.6	90.9±5.0	0.93
Insulin, µU/ml	5.6±3.0	4.4±1.4	0.22
HOMA-IR	1.3±0.6	1.0±0.3	0.22
HbA1c, %	4.7±0.3	4.7±0.2	0.51
Creatinine, mg/dl	0.84±0.10	0.84±0.05	0.85
eGFR, ml/min/m²	91.6±12.2	92.1±6.7	0.91
NT-proBNP, ng/l	18.6±18.0	10.1±3.9	0.15
Urinary acid, mg/l	6.0±0.9	5.4±1.3	0.15
Anaerobic threshold, ml/kg/min	19.0±5.2	29.2±6.6	0.0002
VO₂max, ml/kg/min	43.2±8.0	52.3±6.2	0.0057
CAVI	6.5±0.7	6.2±0.6	0.53
IPAQ score	2318±1605	5310±2869	0.0048

Values are mean ± SD. bpm = beats per minutes, LDL = low-density lipoprotein; HDL = high-density lipoprotein; eGFR = estimated glomerular filtration rate; HOMA-IR = homeostasis model assessment of insulin resistance, NT-proBNP = N-terminal pro brain natriuretic peptides, VO₂max = maximal oxygen intake, CAVI = cardio ankle vascular index, IPAQ = international physical activity questionnaire.
P value denotes significance of unpaired t test between athlete group and healthy control.
doi:10.1371/journal.pone.0061604.t001

Table 2. MRI variables.

	Control group (n = 15)	Athlete group (n = 10)	P value
LV ejection fraction, %	50.6±5.5	48.1±6.3	0.32
LV end diastolic volume, ml	153±16	182±24	0.0011
LV end systolic volume, ml	73±8	95±16	0.0002
Stroke volume, ml	80±14	88±17	0.22
Cardiac output	4.8±0.8	5.2±1.2	0.29
LV myocardial mass, g	120±13	139±16	0.0034
Peak ejection rate, ml/sec	551±206	777±230	0.019
Peak filling rate, ml/sec	619±177	839±250	0.018
Epicardial fat volume, ml	48.8±14.8	38.3±8.2	0.057

Values are mean ± SD. LV = left ventricular.
P value denotes significance of unpaired t test between athlete group and healthy control.
doi:10.1371/journal.pone.0061604.t002

We measured several TG-associated enzymes and proteins, including adiponectin, pre-heparin LPL, apolipoprotein (apo) CII, and apo CIII. No significant difference was observed between the two groups for each parameter (data not shown). One of the major reasons, why these enzyme and proteins were not significantly different, is supposed to the study subjects consisting with healthy lean young men without any metabolic disorder. Myocardial lipid metabolism is regulated by a complex balance between fatty acid supply to the heart, competing energy substrates, energy demand and oxygen supply to the heart, uptake and esterification of fatty acid, and control of mitochondrial functions such as fatty acid oxidation and electron transport chain activity [38]. In addition, epicardial fat, which stores free fatty acid during excessive circulating free fatty acid accumulation and releases fatty acid when energy is needed, is directly connected to the myocardium.

muscle [39]. Therefore, insulin responsiveness and its consequences in the heart may be relatively high in endurance athletes.

A recent study has shown that acute endurance exercise leads to increased myocardial TG content depending on elevated plasma free fatty acid concentrations and the uptake of free acids in the heart. The mechanism is considered to be related to the increased availability of fatty acid during exercise in fasting healthy males [40]. The level of circulating free fatty acids concentration was low in the present study. Thus, fatty acid availability must be relatively low in these individuals. Indeed, myocardial TG content was not reported to change even after exercise in subjects with a suppressed state of free fatty acid synthesis [40]. In addition, endurance training regulates the activity of LPL [41], which provides the major source of free fatty acids derived from TG content lipoproteins. Endurance athletes manifesting physiological LV adaptations may be augmented to drive alterations in fatty acid metabolism on fasting state.
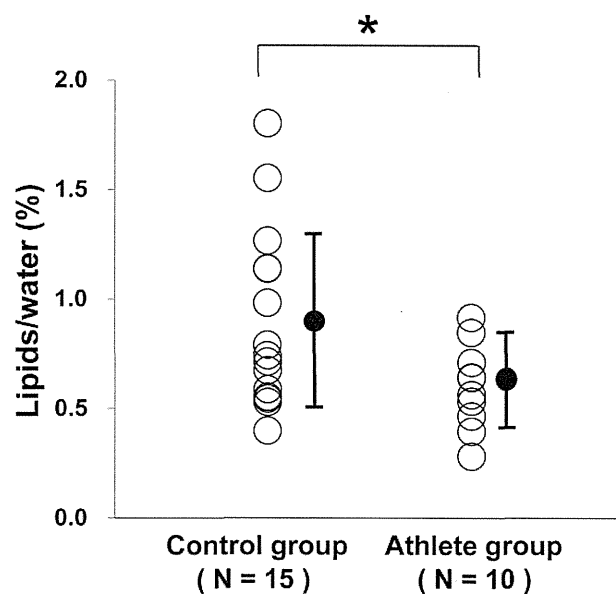

Figure 2. Comparison between myocardial TG content in the control group and the athlete group. * P<0.05 between the two groups.
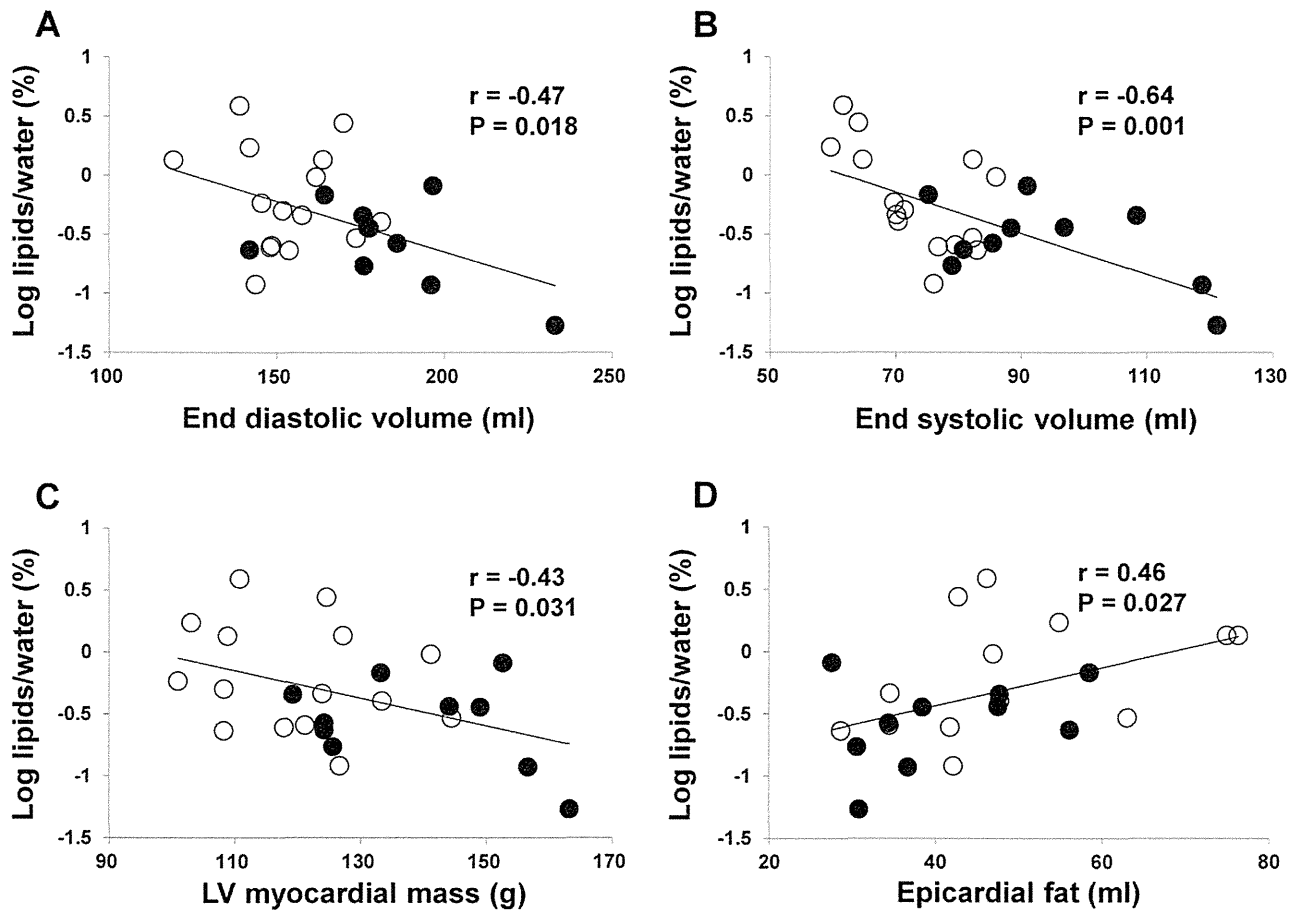doi:10.1371/journal.pone.0061604.g002

Figure 3. Correlations between myocardial TG content and MRI parameters. A: A correlation between myocardial TG content and end-diastolic volume. B: A correlation between myocardial TG content and end-systolic volume. C: Correlation between myocardial TG content and left ventricular (LV) mass. D: Correlation between myocardial TG content and epicardial fat volume. Open circle; control group. Closed circle; athlete group.
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Accordingly, we report a significant positive correlation between epicardial fat volume and myocardial TG content. It has reported that the metabolic rates of lipolysis and lipogenesis are 2-fold higher in epicardial fat than in other fat deposits. Indeed, we detected a negative correlation between epicardial fat volume and EDV, a LV morphology parameter. The precise mechanism underlying the low myocardial TG content in endurance athletes remains elusive. However, the significant positive correlation between epicardial fat volume and myocardial TG content may be related to the increase of utilizing fatty acid in endurance athletes. In our next step, we plan to clarify the impact of exercise on myocardial TG content and LV alterations in endurance athletes.

Limitations

The present study has several limitations. First, this was a single center study with a small sample size, studies of larger sample size are required to confirm these findings. Second, this study included only male subjects. Third, a previous study has demonstrated that a negative relationship between myocardial TG content and cardiopulmonary fitness in obese women [26]. This correlation between myocardial TG content and VO_{2max} was not found in our study. This discrepancy may have resulted from the difference between the subjects in these studies, as in the present study, all subjects of the present study were healthy males without metabolic

disorders. Finally, athlete's heart is considered to be reversible [42], therefore, we will next evaluate the effect of detraining on myocardial TG content.

Conclusions

Low levels of myocardial TG content were observed in endurance athletes and were associated with the morphology of physiological LV alteration. These data suggest that metabolic imaging for measurement of myocardial TG content by ^{1}H-MRS may be a useful technique for noninvasively assessing the "athlete's heart".

Acknowledgments

Author Contributions

Interpreted results of experiments: ES KS YT SA HW RK HD. Prepared figures: ES KS TM. Approved final version of manuscript: ES KS TY SS TM MH YT SA HW RK HD. Conceived and designed the experiments: ES KS TY. Performed the experiments: ES TY SS TM MH. Analyzed the data: ES KS. Contributed reagents/materials/analysis tools: ES KS TY. Wrote the paper: ES KS TY.

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Original article

Impact of diabetes on muscle mass, muscle strength, and exercise tolerance in patients after coronary artery bypass grafting

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Summary
Background: The impact of diabetes mellitus (DM) on muscle mass, muscle strength, and exercise tolerance in patients who had undergone coronary artery bypass grafting (CABG) has not been fully elucidated.
Methods: We enrolled 329 consecutive patients who received cardiac rehabilitation (CR) after CABG (DM group, $n = 178$; non-DM group, $n = 151$) and measured lean body weight, mid-upper arm muscle area (MAMA), and handgrip power (HGP) at the beginning of CR. We also performed an isokinetic strength test of the knee extensor (Ext) and flexor (Flex) muscles and a cardiopulmonary exercise testing at the same time.
Results: No significant differences in risk factors, including age, gender, number of diseased vessels, or ejection fraction were observed between the 2 groups. The levels of Ext muscle strength, peak oxygen uptake, and anaerobic threshold were significantly lower in the DM group than in the non-DM group (all $p < 0.05$). Both peak oxygen uptake and MAMA correlated with Ext and Flex muscle strength as well as HGP (all $p < 0.005$). The MAMA, HGP, and Ext muscle strength were lower in patients who received insulin therapy than in those who did not. Interestingly, fasting glucose levels significantly and negatively correlated with Ext muscle strength.

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Conclusions: These data suggest that DM patients had a lower muscle strength and exercise tolerance than non-DM patients. Moreover, a high glucose level may affect these deteriorations in DM patients after CABG.

Introduction

Individuals with diabetes mellitus (DM) are at an increased risk of coronary artery disease (CAD) and DM patients with CAD have poor prognosis [1]. Indeed, DM patients have a 2—4 times higher risk of developing CAD and mortality due to CAD compared with non-DM patients [2]. DM patients benefit from revascularization techniques such as percutaneous coronary intervention and coronary artery bypass grafting (CABG). However, the benefit is less and the risks and complications are greater in DM patients than in non-DM patients. Previous studies have reported a high incidence of bypass graft dysfunction and mortality even in DM patients who underwent CABG [3].

It is clear that cardiac rehabilitation (CR) has numerous benefits such as modulation of risk factors and prevention of future cardiovascular events [4]. The improvement in peak $\dot{V}O_2$ after CR significantly reduces cardiovascular morbidity and mortality in patients with CAD [5]. However, Savage et al. demonstrated that in more than 20% of the patients who enrolled for CR, there was no improvement in peak $\dot{V}O_2$, and that the diagnosis for DM is negatively associated with the improvement in peak $\dot{V}O_2$ [6]. Vergès et al. reported a significant inverse relationship between fasting blood glucose levels and change in peak $\dot{V}O_2$ in CR participants with DM after acute coronary events [7].

However, the association between muscle mass, muscle strength, and exercise tolerance in patients with or without DM after CABG has not been fully elucidated. The aim of the present study was to investigate the impact of DM on muscle mass, muscle strength, and exercise tolerance in patients who had undergone CABG.

Methods

Subjects

We enrolled 329 consecutive patients who received CR after CABG at the Juntendo University Hospital from July 2002 to February 2005. The patients were divided into 2 groups: patients with DM (DM group, $n = 178$) and patients without DM (non-DM group, $n = 151$) according to the guideline of the Japan Diabetes Society (JDS), including a history of medical treatment, fasting plasma glucose $\geq 126 \, mg/dl$ or casual plasma glucose $\geq 200 \, mg/dl$ and hemoglobin (Hb) A1c (JDS) $\geq 6.1\%$ [8]. All patients participated in CR after 6—8 days of undergoing CABG. All subjects gave written informed consent and the ethical committee of the institution approved this study.

Measurements

We assessed body composition, muscle strength, and exercise tolerance at the beginning of CR. Anthropometric parameters were assessed using body mass index and waist circumference. Triceps skin-fold thickness of the dominant hand was measured in millimeters using a caliper, while the mid-upper arm circumference was measured in centimeters using a tape measure. The mid upper-arm muscle area (MAMA) was calculated according to the standard formula [9]. Moreover, we measured the handgrip power (HGP) of the dominant hand. The percentages of body fat and lean body weight were measured by a BOD POD® (Life Measurement, Inc., Concord, CA, USA), as we described previously [10,11]. In addition, thigh muscle power was measured using the Cybex770 system (Cybex Division of Lumex, Ronkonkoma, NY, USA), as reported earlier [10,11]. The isokinetic peak torques of the knee extensor (Ext) and flexor (Flex) muscles were measured at 60°/s; these were adjusted by body weight according to the following formula: strength (Nm) $\times$ 100/body weight (kg). Patients underwent ergometer testing (Corival 400, Lobe B.V., Groningen, Netherlands) using an expiratory gas analysis machine (Vmax-295, SensorMedics Co., Yorba Linda, CA, USA) to measure peak oxygen consumption (peak $\dot{V}O_2$) and the anaerobic threshold (AT). After a rest period, a warm-up was performed for a few minutes at 20 W, followed by ramp loading (15 W/min) until subjective exhaustion, progressive angina, ST-segment depression ($\geq 2 \, mm$), or sustained tachyarrhythmia. The AT point was determined by the "V-slope" method.

Statistical analyses

Results are expressed as the mean ± standard deviation and were analyzed using the StatView software (Version 5.0J for Windows, SAS Institute, Cary, NC, USA). Comparisons between the DM and non-DM groups were performed by a two-tailed Student's t-test. Correlation coefficients (r) were determined by linear regression analysis. Statistical significance of the correlation coefficients was determined by the method of Fisher and Yates. A p-value of less than 0.05 was considered significant.

Results

Characteristics of the study subjects

Clinical characteristics and anthropometric parameters of the subjects are presented in Tables 1 and 2. One hundred and seventy-eight patients (54%) were diagnosed as having DM. No significant differences in risk factors, including age, gender, number of diseased vessels, ejection fraction, and physiological variables were observed between the DM and non-DM groups. Three hundred and twenty-five patients (99%) received complete revascularization using the off-pump operation. Eight patients (4%) who had undergone re-CABG were in the DM group. No significant differences were observed between the 2 groups for the concomitant use of drugs such antiplatelet agents, calcium-channel blockers, β-blockers, angiotensin-converting enzyme inhibitors,

Table 1 Clinical characteristics of the study subjects.

	DM	Non-DM	p value
N	178	151	
Age (year)	64.7 ± 9.2	65.8 ± 9.2	NS
Male (%)	143 (80)	120 (79)	NS
Hypertension (%)	116 (66)	111 (74)	NS
Dyslipidemia (%)	109 (62)	108 (72)	NS
Current smoker (%)	91 (54)	72 (49)	NS
Familial history (%)	40 (24)	33 (23)	NS
Fasting blood glucose (mg/dl)	159 ± 63	108 ± 24	<0.01
HbA1c (%)	7.1 ± 1.3	5.1 ± 0.4	<0.01
LDL-C (mg/dl)	115 ± 34	110 ± 41	NS
HDL-C (mg/dl)	47 ± 13	48 ± 13	NS
Triglyceride (mg/dl)	140 ± 77	143 ± 76	NS
Creatinine (mg/dl)	1.3 ± 1.7	1.2 ± 1.9	NS
C-reactive protein (mg/dl)	0.5 ± 1.5	0.3 ± 0.3	NS
History of MI (%)	38 (28)	31 (25)	NS
History of PCI (%)	4 (2)	0 (0)	NS
History of previous CABG (%)	8 (4)	0 (0)	NS
Diseased vessels			
LMT (%)	36 (20)	21 (14)	NS
3VD (%)	103 (58)	89 (59)	NS
1–2VD (%)	39 (22)	41 (27)	NS
Ejection fraction (%)	57.3 ± 15.2	59.1 ± 16.3	NS
Off-pump CABG (%)	174 (98)	151 (100)	NS

Data are presented as the mean value ± SD. DM, diabetes mellitus; HbA1c, hemoglobin A1c; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; MI, myocardial infarction; PCI, percutaneous coronary intervention; CABG, coronary arterial bypass grafting; LMT, left main trunk; VD, vessel disease.

angiotensin II receptor blockers, and statins. In the DM group, 110 patients (61%) and 55 (31%) patients were treated with oral anti-diabetic agents and insulin, respectively.

Exercise tolerance and muscle strength

The exercise tolerance and muscle strength of the 2 groups are presented in Table 3. The levels of peak $\dot{V}O_2$ (12.5 ± 3.7 ml kg^{-1} min^{-1} vs. 13.7 ± 4.0 ml kg^{-1} min^{-1}; $p = 0.01$) and AT (8.3 ± 1.6 ml kg^{-1} min^{-1} vs. 8.8 ± 2.1 ml kg^{-1} min^{-1}, $p = 0.02$) were significantly lower in the DM group than in the non-DM group. Ext muscle strength was significantly lower in the DM group than in the non-DM group (131 ± 40 Nm kg^{-1} × 100 vs. 146 ± 45 Nm kg^{-1} × 100,

$p < 0.01$). No significant differences in HGP (27.7 ± 9.0 kg vs. 29.5 ± 9.0 kg, NS) were observed between the 2 groups. Peak $\dot{V}O_2$ values were correlated with Ext muscle strength of thigh ($r = 0.49$, $p < 0.005$) (Fig. 1A) and HGP ($r = 0.44$, $p < 0.005$) (Fig. 1B); MAMA values were correlated with Ext muscle strength of thigh ($r = 0.42$, $p < 0.005$) (Fig. 2A) and HGP ($r = 0.64$, $p < 0.005$) (Fig. 2B). The same trends were observed in the DM and non-DM patients (Figs. 1C–F, 2C–F).

Diabetes mellitus and MAMA

To assess the effects of insulin treatment, we divided DM patients into the following 2 groups: DM patients undergoing insulin therapy (insulin-treated DM group) and DM patients

Table 2 Comparison of anthropometric parameters between the DM and non-DM groups.

	DM	Non-DM	p value
Body mass index (kg/m^2)	23.3 ± 2.7	23.2 ± 2.7	NS
Lean body weight (kg)	49.2 ± 8.6	48.0 ± 4.1	NS
Waist circumference (cm)	84.8 ± 8.0	84.0 ± 8.0	NS
Thigh circumference (cm)	47.0 ± 6.7	48.1 ± 4.1	NS
Arm forced circumference (cm)	28.3 ± 2.7	28.7 ± 2.5	NS
Triceps skinford thickness (mm)	10.9 ± 6.0	10.7 ± 4.0	NS
Mid-upper arm muscle circumference (cm)	24.9 ± 2.6	25.4 ± 2.5	NS
MAMA (cm^2)	50.0 ± 10.0	52.0 ± 10.0	NS

Data are presented as the mean ± SD. DM, diabetes mellitus; MAMA, mid-upper arm muscle area.

Table 3 Comparison of exercise tolerance and muscle strength between the DM and non-DM groups.

	DM	Non-DM	p value
Baseline			
SBP (mmHg)	134 ± 23	128 ± 19	<0.01
HR (min^{-1})	87 ± 13	90 ± 13	0.04
PRP (mmHg min^{-1})	11673 ± 2333	11634 ± 2280	NS
Anaerobic threshold			
Anaerobic threshold (ml kg^{-1} min^{-1})	8.3 ± 1.6	8.8 ± 2.1	0.02
Workload (W)	32 ± 12	34 ± 16	NS
HR (min^{-1})	101 ± 13	105 ± 13	0.01
Peak exercise			
Peak VO_2 (ml kg^{-1} min^{-1})	12.5 ± 3.7	13.7 ± 4.0	0.01
Workload (W)	73 ± 22	71 ± 27	NS
RER	1.06 ± 0.14	1.07 ± 0.14	NS
SBP (mmHg)	180 ± 31	181 ± 30	NS
HR (min^{-1})	117 ± 17	121 ± 17	0.02
PRP (mmHg min^{-1})	21177 ± 5097	22088 ± 5427	NS
Peak HR—resting HR (min^{-1})	30 ± 13	32 ± 15	NS
$\Delta VO_2 / \Delta WR$ (ml min^{-1} W^{-1})	8.4 ± 2.6	8.6 ± 2.2	NS
Muscle strength			
Knee extension (Nm kg^{-1} × 100)	131 ± 40	146 ± 45	<0.01
Knee flexion (Nm kg^{-1} × 100)	73 ± 23	79 ± 28	NS
Hand grip power (kg)	27.7 ± 9.0	29.5 ± 9.0	NS

Data are presented as the mean value $\pm$ SD. DM, diabetes mellitus; SBP, systolic blood pressure; HR, heart rate; PRP, pressure rate product; RER, respiratory exchange ratio; WR, work rate.

without insulin therapy (non-insulin-treated DM group). No significant differences in risk factors, number of diseased vessels, prevalence of re-CABG, and ejection fraction were observed between the non-insulin-treated DM group and the insulin-treated DM group. The insulin-treated DM group had a significantly longer duration of DM history than the non-insulin-treated DM group (17.7 ± 9 years vs. 11.7 ± 10 years, $p < 0.01$). The prevalence of microvascular complications, including retinopathy, nephropathy, and neuropathy, tended to be higher in the insulin-treated DM group than in the non-insulin-treated DM group (86% vs. 67%, $p = 0.09$). MAMA levels were significantly lower in the insulin-treated DM group than in the non-insulin-treated DM group (45.9 ± 9.8 cm^2 vs. 51.9 ± 9.7 cm^2, $p < 0.01$). The insulin-treated DM group had a low thigh muscle strength (121.5 ± 29 Nm kg^{-1} × 100 vs. 135.4 ± 42 Nm kg^{-1} × 100, $p = 0.06$) and HGP (25.6 ± 8.0 kg vs. 28.9 ± 8.0 kg, $p = 0.05$). In addition, a significant inverse relationship was observed between fasting blood glucose and Ext muscle strength of thigh in the DM group ($r = -0.26$, $p < 0.005$) (Fig. 3). A weak but significant inverse relationship was also observed between HbA1c and Ext muscle strength of thigh ($r = -0.17$, $p < 0.05$).

Discussion

In the present study, we demonstrated that: (1) DM patients had a significantly lower exercise tolerance and muscle strength compared with non-DM patients; (2) exercise tolerance and muscle mass correlated with muscle strength; and (3) fasting glucose levels significantly and negatively correlated with muscle strength in patients who received CR after CABG. These data suggest that a high glucose level

may affect these deteriorations in DM patients after CABG. A relationship between muscle strength and peak VO_2 has already been reported [12,13]. However, to the best of our knowledge, this is the first report demonstrating the impact of DM on muscle mass, muscle strength, and exercise tolerance in patients at the beginning of CR after CABG.

The reason why DM patients have low levels of exercise tolerance and muscle strength should be discussed. Tesfamariam et al. showed that the dysfunction of endothelium-dependent relaxation associated with exposure to elevated glucose levels is due to the increased production of vasoconstrictor prostanoids by the endothelium as a consequence of protein kinase C activation [14]. Previous studies have demonstrated that metabolisms of both glucose and fatty acids by skeletal muscle as well as the bioenergetic capacity of skeletal muscle mitochondria are impaired in DM patients [15]. These proposed mechanisms may explain the data in the present study because a significant inverse relationship was observed between fasting blood glucose levels and thigh muscle strength in the DM group (Fig. 3). Recently, Womack et al. showed that DM patients with microvascular complications have impaired capillary recruitment to contractile exercise [16]. In the present study, the prevalence of microvascular complications tended to be higher in the insulin-treated DM group than in the non-insulin-treated DM group. This may also be one of the mechanisms by which thigh muscle strength and HGP were significantly lower in the insulin-treated DM group than in the non-insulin-treated DM group. Low exercise tolerance in DM patients may be caused by sensorineural and autonomic dysfunction. An impaired heart rate response to exercise has been regarded as chronotropic incompetence and is seen in DM patients with impaired
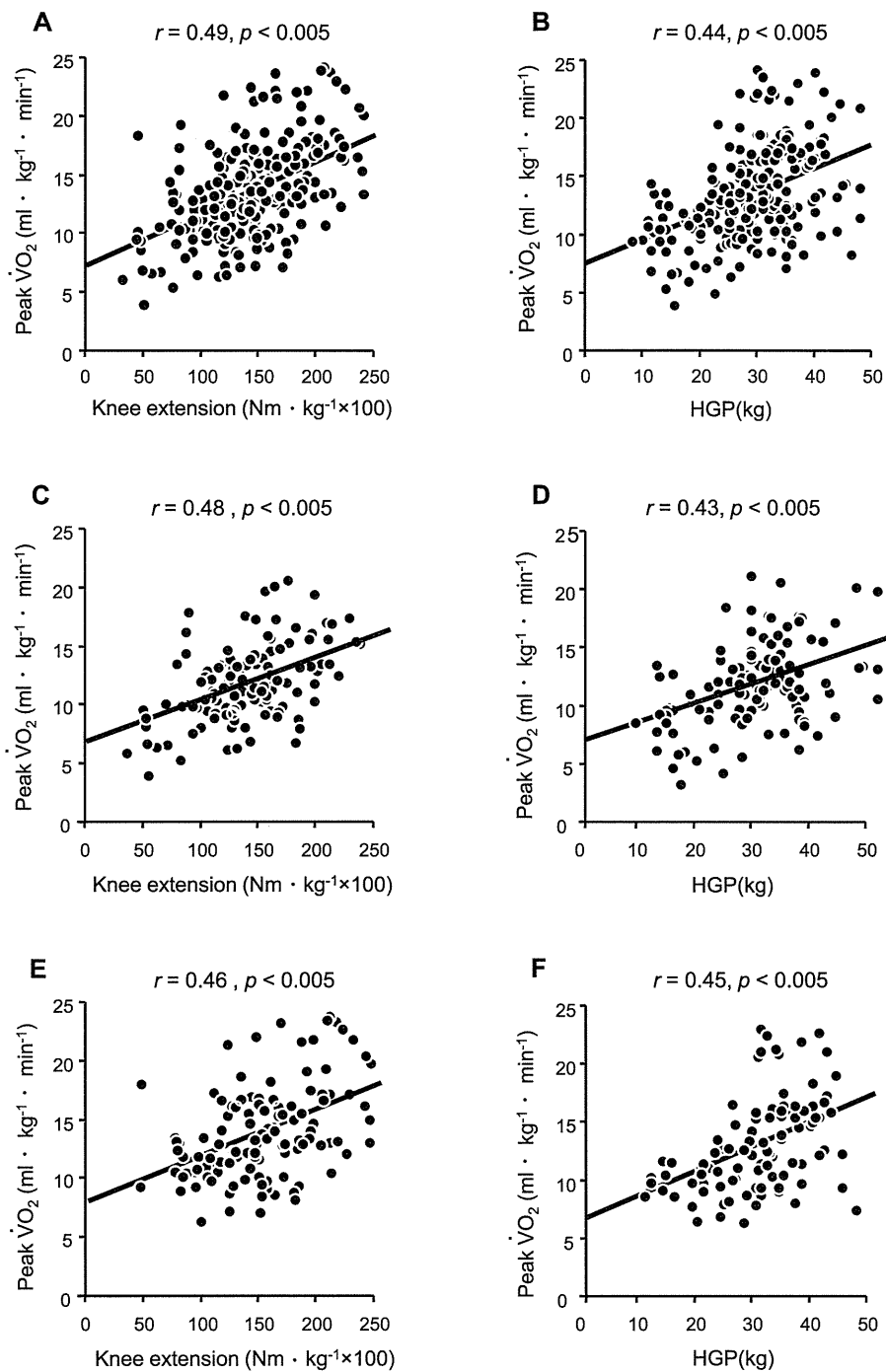
Figure 1 Correlations between exercise tolerance and muscle strength. Peak $\dot{V}O_2$ correlated with extensor muscle strength of thigh ($r=0.49$, $p<0.005$) (A), and HGP ($r=0.44$, $p<0.005$) in all patients (B) ($n=259$). Peak $\dot{V}O_2$ correlated with extensor muscle strength of thigh ($r=0.48$, $p<0.005$) (C), and HGP ($r=0.43$, $p<0.005$) in the DM group (D) ($n=128$). Peak $\dot{V}O_2$ correlated with extensor muscle strength of thigh ($r=0.46$, $p<0.005$) (E), and HGP ($r=0.45$, $p<0.005$) in the non-DM group (F) ($n=131$). HGP, hand grip power; DM, diabetes mellitus.

exercise capacity. A previous study showed that low exercise capacity may be an impaired chronotropic response to exercise in DM patients with acute myocardial infarction [17]. In the present study, the heart rate values at peak $\dot{V}O_2$ and AT were significant lower in the DM group than in the non-DM group; however, the increased changes in heart rate were identical for the two groups (Table 3).

Therefore, sensorineural and autonomic dysfunction may not have affected exercise intolerance in the DM group. The changes in sympathetic nervous activity (e.g. plasma catecholamine levels and R—R interval variability on an electrocardiogram) would be assessed in the subsequent step. Besides, a delta-$\dot{V}O_2$/delta-WR ($\Delta\dot{V}O_2/\Delta$WR) is determined by the rate of increase in cardiac output and the rate of
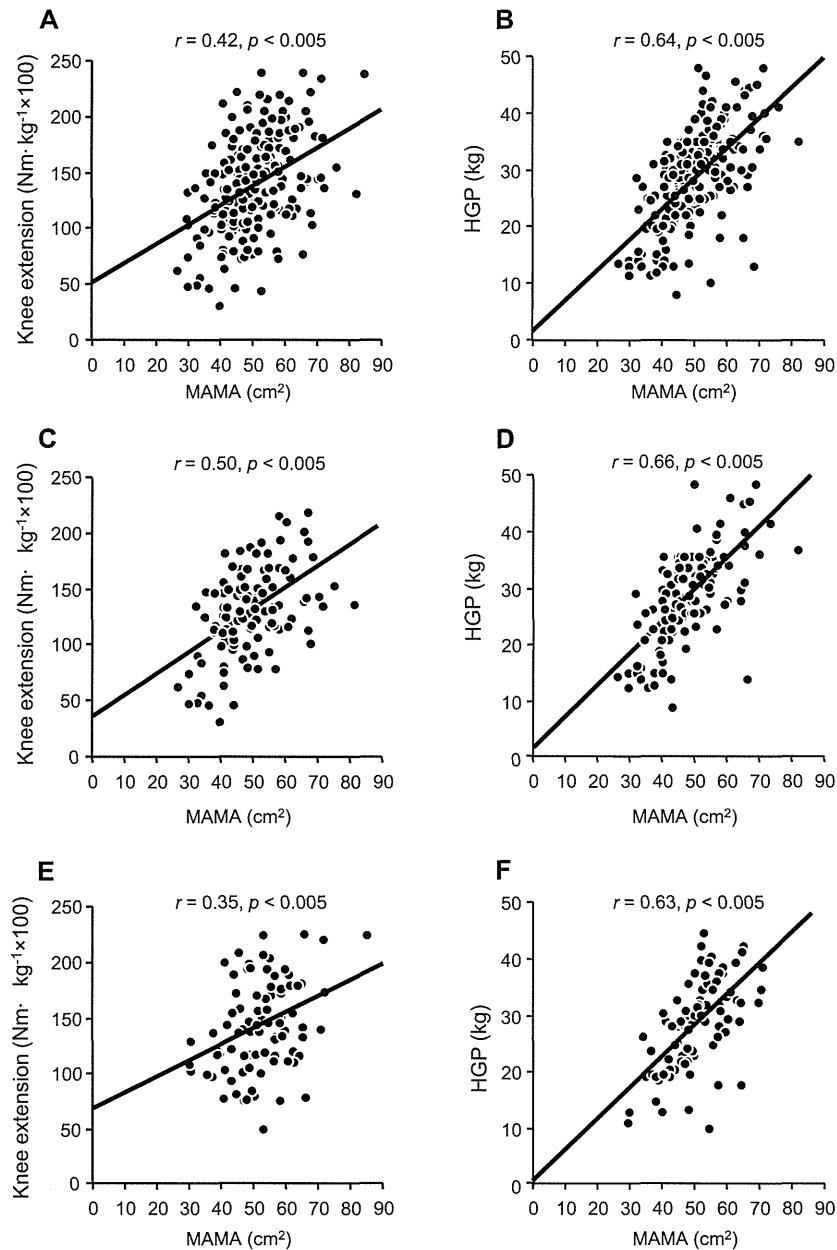
Figure 2   Correlations between muscle strength and muscle mass. MAMA correlated with extensor muscle strength of thigh ($r = 0.42$, $p < 0.005$) (A), and HGP ($r = 0.64$, $p < 0.005$) (B) in all patients ($n = 201$). MAMA correlated with extensor muscle strength of thigh ($r = 0.50$, $p < 0.005$) (C), and HGP ($r = 0.66$, $p < 0.005$) (D) in DM patients ($n = 108$). MAMA correlated with extensor muscle strength of thigh ($r = 0.35$, $p < 0.005$) (E), and HGP ($r = 0.63$, $p < 0.005$) (F) in non-DM patients ($n = 93$). MAMA, mid-upper arm muscle area; HGP, hand grip power; DM, diabetes mellitus.

difference in arterial mixed venous oxygen during incremental exercise [18]. Comparing with the non-DM group, $\Delta \dot{V}O_2 / \Delta WR$ values were low but not significant in the DM group (Table 3).

The present study demonstrated that MAMA correlated with thigh muscle strength and HGP and MAMA levels were significantly lower in the insulin-treated DM group than the non-insulin-treated DM group. Chronic hyperglycemia leads to the production of amadori products through nonenzymatic glycation reactions between glucose and reactive amino groups of serum proteins [19]. These products

undergo further irreversible reactions to form advanced glycation end products that promote insulin resistance as well as trigger inflammation and secretion of cytokines and growth factors, which leads to amplification or progression of various diseases including diabetic vascular complications (metabolic memory) [20]. In the present study, the insulin-treated DM group had a significantly longer duration of DM history than the non-insulin-treated DM group. The loss of muscle mass may be caused by chronic hyperglycemia, the so-called negative legacy effect, particularly in the insulin-treated DM group. A recent study demonstrated that a low
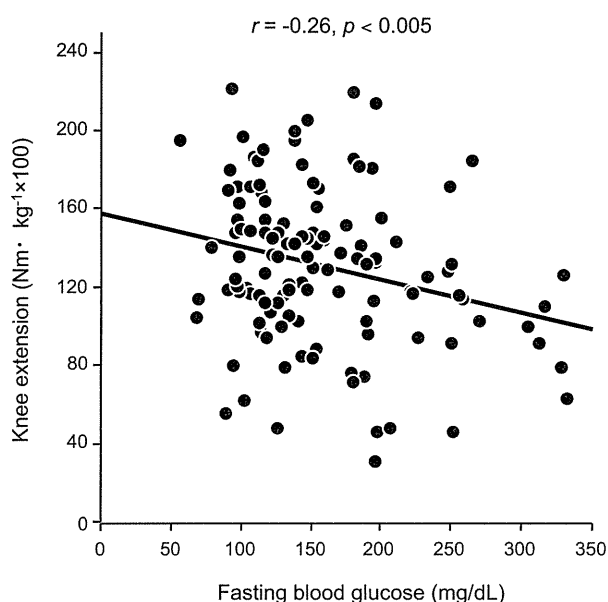
$r = -0.26, p < 0.005$

Figure 3 Correlation between fasting glucose levels and extensor muscle strength of thigh. A significant inverse relationship was observed between fasting blood glucose levels and extensor muscle strength of thigh in the diabetes mellitus group ($r = -0.26$, $p < 0.005$) ($n = 120$).

level of arm muscle area was an independent risk factor for the 2-year mortality in a cohort of community dwelling Japanese elderly [21]. We would like to further clarify whether the levels of arm muscle area before and after CR can predict morbidity and mortality in DM patients after CABG.

CR is class I recommendation in most contemporary cardiovascular clinical practice guidelines. Exercise tolerance has proven to be the strongest predictor of the risk of death among subjects with and without known cardiovascular disease [22]. Vergès et al. reported that the benefit of CR on exercise capacity is significantly lower in DM patients than non-DM patients and the response to CR was influenced by blood glucose levels [7]. A previous study demonstrated that exercise increases the activity of AMP-activated protein kinase in muscle, which in turn, promotes translocation of the glucose transporter-4 from the cytosol to the plasma membrane, increases insulin-independent glucose uptake by muscle, and improves muscle insulin resistance by a reduction of intramyocellular lipids [23]. Therefore, it is necessary to investigate the effects of CR on muscle mass, muscle strength, exercise capacity, and long-term outcome.

The present study has some limitations. First, this was a single-center study with a small sample size. Studies with larger sample sizes can confirm these results. Secondly, we performed a cardiopulmonary exercise test at the beginning of phase I CR (6—8 days after CABG). Therefore exercise tolerance and muscle strength might be attenuated by confounding factors. However, no significant differences in the clinical characteristics were observed between the DM group and the non-DM group. Thirdly, we enrolled patients who received CR after CABG. Therefore, the results of the present study may not be representative of all DM patients with CAD. Finally, this was a cross-sectional study. As dis-

cussed above, the clinical importance of muscle parameters and exercise tolerance prospectively as well as the effects of CR on muscle mass, muscle strength, exercise capacity, and future cardiovascular events in DM patients after CABG must be investigated.

Conclusions

DM patients had a lower muscle strength and exercise tolerance than non-DM patients at the beginning of CR after CABG. Moreover, a high glucose level may affect these deteriorations in DM patients after CABG. Further studies are required to assess whether CR would ameliorate these deteriorations and improve the clinical prognosis in DM patients after CABG.

Acknowledgement

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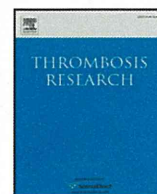
Regular Article

Patient Factors against Stable Control of Warfarin Therapy for Japanese Non-valvular Atrial Fibrillation Patients

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ABSTRACT

Introduction: Effectiveness and safety of warfarin therapy for non-valvular atrial fibrillation (NVAF) patients are strongly associated with its stability presented such as time in therapeutic range (TTR) of PT-INR. However, the factors that affect TTR have not been fully elucidated in Japan where majority of patients are controlled within the range of 1.6-2.6 of PT-INR irrespective of the age.
Methods: We retrospectively analyzed 163 NVAF patients taking warfarin to determine the factors that affect TTR including metabolic enzymes polymorphisms after TTR calculation with both the standard PT-INR range and the actual control range of 1.6-2.6.
Results: Overall TTR calculated using Japanese Guideline was 69.7 ± 25.1% (<70 and ≥70 years; 49.6 ± 24.8% and 77.8 ± 20.3%, respectively). After confirming that PT-INR values in patients <70 years distributed in the same range as in those ≥70 years, as in a Japanese large cohort, we recalculated TTR of those <70 years with 1.6-2.6 of PT-INR and found that it was 79.5 ± 20.1%. Poor control of this new TTR were significantly associated with the lower height, the higher serum creatinine, the lower creatinine clearance, female gender, and presence of congestive heart failure, ($p < 0.05$ respectively). Multivariate analysis revealed female gender and presence of congestive heart failure as independent predictor of the lower TTR ($p < 0.05$, $p < 0.01$, respectively). Polymorphism of CYP2C9 and VKORC1 were related to the dosage of warfarin but not determinant of TTR.
Conclusions: When evaluated using a range of PT-INR actually used in Japan, TTR is generally well controlled and female gender and presence of congestive heart failure significantly affected the poorer TTR control.

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Introduction

Atrial fibrillation (AF) is a common and rapidly increasing arrhythmia in the current high-aged society. AF is highly associated with substantial morbidity and mortality particularly due to cerebral thromboembolism [1]. Though novel anti-coagulating agents have been recently developed especially for those with non-valvular AF (NVAF), warfarin still remains the most common and important anticoagulants used to reduce the risk of stroke in AF patients. The meta-analysis by Hart and co-workers reported that adjusted-dose warfarin reduced the risk of stroke by 64% (49%–74%) in NVAF

patients [2]. Though the pharmacokinetics of warfarin is profoundly affected by many factors, it has not been fully understood what affect the actual control of international normalized ratio of prothrombin time (PT-INR) substantially. Recently, it has been known that the outcome of NVAF patients on warfarin depends on the length of the adequate PT-INR control presented as time in therapeutic range (TTR) [3,4]. It is reported that if the control of TTR value is <40%, the risk of stroke will increase even more than without using warfarin [4]. As many factors are suspected to affect TTR control but not determined yet, and to elucidate these factors should be of great importance, we decided to examine the relationship between the various factors and TTR among Japanese population. Particularly, as previous study by Okumura and co-workers reported that the dosage of warfarin significantly related to TTR value [5], we also investigated the relationship between warfarin dosage and polymorphism of

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metabolic enzymes (CYP2C9, CYP2C19, CYP4F2,and VKORC1), then we investigated the relationship between TTR and polymorphism of metabolic enzymes to evaluate final influence of these polymorphisms [6–8].

After PT-INR range of 2.0-3.0 has been established as an appropriate therapeutic range according to the results of stroke prevention trials in AF [9] and cohort studies in Europe and North America [10], ACC/AHA/ESC guidelines recommend this PT-INR range for anticoagulant therapy with warfarin in NVAF patients. In Japan, as Yasaka et al found that the major ischemic or hemorrhagic events occurred in elderly Japanese NVAF patients when they were controlled out of 1.6-2.6 of PT-INR range, this PT-INR range has been generally considered as optimal for elderly patients ≥ 70 years to prevent such events [11] and accordingly it was documented in the Japanese Guidelines for Pharmacotherapy of Atrial Fibrillation [12]. Previous study from Japan reported that the control of TTR is good in the patients ≥ 70 years but poor in those <70 years [5,13]. This fact seems to have derived from the fact that most Japanese cardiologists tend to keep PT-INR within 1.6-2.6 even in those <70 years. The result a recent large Japanese cohort study backed up this notion [13]. Therefore, it seems particularly important to know the actual range of PT-INR of the controlling doctors to elucidate the real influence of each potential factor on TTR in NVAF patients.

Methods

From July 2011 to March 2012, we collected data of 163 NVAF outpatients ≥ 40 years undergoing warfarin therapy for ≥ 6 months, who had been followed up at four institutes and retrospectively analyzed the relationship between TTR and the candidate factors including age, height, body weight, gender, serum creatinine, creatinine clearance (CCr; Cockcroft-Gault method), serum albumin, components of CHADS2 score (congestive heart failure, hypertension, age >75 years, diabetes mellitus, and prior stroke/transient ischemic attack), dosage of warfarin at enrollment, co-administration of antiplatelet drug. Patient was eligible only after confirming that at least 2 points were kept within appropriate PT-INR range in order to exclude those within unstable initiation period of warfarin therapy.

All the attending physician were familiar to the Japanese Guidelines for Pharmacotherapy of Atrial Fibrillation (JCS2008); that is, between 2.0 and 3.0 for patients <70 years and between 1.6 and 2.6 for those ≥ 70 [12]. However, the actual dosage of warfarin was determined by the discretion of each physician. Administration of warfarin in patients with low CHADS2 score [14] of 0 or 1 was left to the decision of the attending physician.

TTR was determined using the method of linear interpolation between consecutive PT-INR values in each patient reported by Rosendaal method [3,4,15] We also investigated the relationship between warfarin dosage and polymorphism of metabolic enzymes (CYP2C9, CYP2C19, CYP4F2,and VKORC1), then we investigated the relationship between TTR and polymorphism of metabolic enzymes to evaluate the final influence of these polymorphisms [6–8]. Genetic variation analysis was carried out according to the previous report [8]. In brief, genomic DNA was extracted from whole blood. The VKORC1-1639 G > A, CYP2C9*2, CYP2C9*3, CYP2C19*2, CYP2C19*3 and CYP4F2-1347 C > T polymorphisms were determined using the PCR–restriction fragment length polymorphism method. Nine patients were dropped out because of informed consent or technical error, and then we examined 154 patients in genetic variability analysis.

All data are expressed as mean ± standard deviation. For comparison of TTR between 2 groups Mann Whitney test was used. For comparison of TTR among 3 or more groups, 1-way analysis of variance (Kruskal-Wallis test) was used followed by post-hoc Dunns test. For correlation of TTR with the candidate factors, we performed univariate-analyses using Spearman rank correlation test, then multiple stepwise regression analysis using JMP Version 9 was

performed to detect the independent predictors of TTR. In order to avoid inclusion of factors that are strongly mutually correlated, we excluded the factors such as creatinine clearance, age >75 years which are components of CHADS2 score, from the multivariate analysis. Thus, height, body weight, gender, creatinine, congestive heart failure, and hypertension, which p values were within 0.1 by univariate-analyses, were used in multiple stepwise regression analysis (backward elimination method). P < 0.05 was considered significant.

The study protocol was approved by the ethics committee of each institution and written informed consent was given by each patient.

Results

Patients' Clinical Characteristics

There were 101 male and 62 female patients, and their mean age was 74.4 ± 8.8 years, ranging from 49 to 93. Mean warfarin dose administered at the enrollment was 2.9 ± 1.2 mg/day, ranging from 1.0 to 10 mg/day. Antiplatelet drug were co-administrated in 25 patients (15.3%) (Table 1).

Status of PT-INR Control and Influential Factors

Averaged TTR value under current Japanese Guideline was 69.7 ± 25.1%, (49.6 ± 24.8% and 77.8 ± 20.3%, <70 and ≥70 years, respectively, p < 0.0001) (Fig. 1A,B). We analyzed the factor that influenced TTR value and found that age was only and strongly associated with TTR value (p < 0.0001). However, we noticed that PT-INR in patients <70 years distributed within the same range and with a same shape as those in patient ≥70 years (Fig. 2) as was suggested by a large Japanese cohort study [13]. The average of PT-INR values in patients <70 years and ≥70 years were 2.1 ± 0.6, and 2.0 ± 0.6, respectively. As shown in Fig. 1C, TTR distributed in the very high range even in patients <70 years when TTR was calculated using the "actual" PT-INR

Table 1
Characteristics of the patients.

Total n (%)	163
Age	74.4 ± 8.8
<70 years	47(28.8%)
≥70 years	116(71.2%)
Gender Male	101(62%)
Female	62(38%)
Body weight (kg)	58.8 ± 11.2
Creatinine (umol/L)	88.4 ± 35.4
Serum albumin (mg/L)	42 ± 3
Creatinine clearance (ml/min)	58. ± 24.6
CHADS2 score	
0	26(15.9%)
1	61(37.4%)
2	41(25.2%)
3	24(14.7%)
4	8(4.9%)
5	3(1.8%)
6	0(0%)
Congestive heart failure	13(8.0%)
Hypertension	78(47.9%)
Age (≥75 years old)	89(54.6%)
Diabetes mellitus	44(27.0%)
Stroke	22(13.5%)
TTR[§](guideline)	69.7 ± 25.1%
<70 years old	49.6 ± 24.8%
≥70 years old	77.8 ± 20.3%
TTR (1.6-2.6)	79.5 ± 20.1%
Warfarin dosage (mg)	2.9 ± 1.2
Co-administration of antiplatelet agent	25(15.3%)

Data are expressed as means ± SD.
§ TTR: time in therapeutic range.