Table 3 Potential risk factors for diabetes

	Multivariable adjusted hazard ratio*	(95% CI)
Age (years)	0.56	(0.27–1.15)
Year of graduation	0.67	(0.32–1.39)
BMI (kg/m^2)	1.17	(0.99–1.38)
Smoker (yes/no)	0.59	(0.23–1.50)
College sports club participation (yes/no)	0.80	(0.06–10.16)

Estimates shown as median (IQR) or number (%) and Multivariable adjusted hazard ratio (95% CI).
All items refer to the data of college age subjects.
*Cox proportional hazards models adjusted for 1,500-m endurance run time and all items in the table.

were 1.00 (reference), 0.33 (0.12–0.91) and 0.24 (0.07–0.83). After further adjustment for age, year of graduation, BMI, smoking, and college sports club participation, there remained an inverse association between diabetes risk and cardiorespiratory fitness (p =0.03 for trend). The adjusted hazard ratios for the incidence of type 2 diabetes by cardiorespiratory fitness category (low to high) were 1.00 (reference), 0.40 (0.14–1.13), and 0.26 (0.07–1.00). Overall, men in the highest cardiorespiratory fitness group had a 74% lower risk of developing type 2 diabetes than men in the lowest cardiorespiratory fitness group.

Discussion

In this study, we investigated the relationship between cardiorespiratory fitness at the time of college and the development of type 2 diabetes among Japanese male athletes. Our results show that having a low cardiorespiratory fitness level increased the risk of type 2 diabetes among Japanese male athletes. This result is similar to what had been previously shown among the general Japanese population [7].

To our knowledge, epidemiological studies of fitness and diabetes risk in athletes have previously only been conducted among Caucasian populations. There are reports that former top-level athletes have a low prevalence of type 2 diabetes compared with controls of similar age [8-11]. In particular, former male endurance athletes, such as long-distance runners and cross-country skiers, were shown to have a low prevalence of type 2 diabetes [11]. Although cross-sectional studies have primarily been used to investigate this link, it has been suggested that strength training and high cardiorespiratory fitness specifically contribute to the prevention of type 2 diabetes in athletes.

The Harvard Alumni Study, a long-term cohort study, reported that a low vital capacity at entrance age predisposed subjects to type 2 diabetes later in life [12]. Carnethon et al. also found a significant inverse relationship between cardiorespiratory fitness levels and the incidence of type 2 diabetes in 18- to 30-year-olds [13]. These studies, conducted among Caucasian non-athletes, reported an inverse relationship between cardiorespiratory fitness at a young age and the incidence of type 2 diabetes. The present study indicates that high cardiorespiratory fitness is protective against type 2 diabetes not only in the general population, but also among athletes.

Some plausible mechanisms have been proposed for the link between low cardiorespiratory fitness and diabetes risk; for example, the fact that individuals with low cardiorespiratory fitness tend to have low insulin sensitivity. This positive relationship between the rate of glucose metabolism and maximal oxygen consumption was demonstrated by Sato et al. [14]. Ivy and Kuo also reported that individuals with lower cardiorespiratory fitness levels have fewer glucose transporters than more fit individuals [15].

The cardiorespiratory fitness of athletes is presumably greater than that of the general population. However, the rate of type 2 diabetes in all subjects in the present study was equal to the rate among high cardiorespiratory

Table 4 Adjusted hazard ratio for diabetes according to cardiorespiratory fitness level

	Cardiorespiratory fitness level, tertiles			p for Trend
	Low	Medium	High	
Number of subjects	189	186	195	
Person-years of follow-up	4817	4867	4892	
Diagnosed type 2 diabetes#	14	5	3	
Rate per 10,000 parson-years	29.1	10.3	6.1	
Age adjusted hazard ratio (95% CI)	1.00 (Reference)	0.33 (0.12–0.91)	0.24 (0.07–0.83)	0.01
Multivariable adjusted hazard ratio* (95% CI)	1.00 (Reference)	0.40 (0.14–1.13)	0.26 (0.07–1.00)	0.03

*Cox proportional hazards models adjusted for age, year of graduation, BMI, smoking and college sports club participation at college age.
#Diabetes was diagnosed by physician.

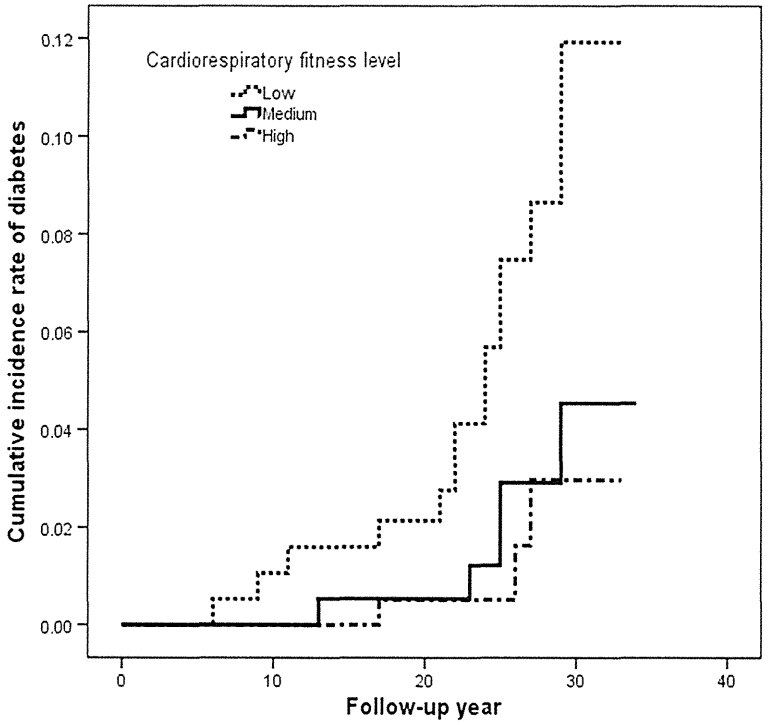

Figure 2 Cumulative incidence rate curve for type 2 diabetes during follow-up, according to cardiorespiratory fitness level.

fitness groups in the Japanese male general population [7]. Therefore, it is appropriate to investigate this topic among athletes. In addition, this was a cohort study of Japanese athletes. Previous studies investigating the relationship between cardiorespiratory fitness and the development of diabetes in athletes were limited to cross-sectional studies and were conducted only among Caucasian populations. Because the incidence of type 2 diabetes differs among ethnic populations; the association of fitness with diabetes risk may also differ among ethnic groups.

Several limitations of this study need to be discussed. First, the subjects are not representative of all Japanese athletes. Only male college alumni were studied. However, these students came from all over Japan, and were selected for admission by an entrance examination of motor skills. In addition, men without both a cardiorespiratory fitness test at college age and a follow-up questionnaire were excluded. These exclusions limit the generalizability of the study, but not its validity. In this study, female alumni were also excluded because they were not registered at the college in 1971. However, we are reflecting on the need to investigate and validate similar data for women. Another limitation is that cardiorespiratory fitness was measured by a 1,500-m endurance run test rather than by laboratory measurements of values such as maximal oxygen consumption. Although the 1,500-m endurance run is a field test, an inverse relationship has been demonstrated between 1,500-m endurance run times and maximal oxygen consumption [16-18]. It was also reported that distance runs over 1 km adequately assess cardiorespiratory capacity [19,20], and that the reliability of field tests has been established for physical education majors [21]. Finally, self-selection bias was possible because the subjects who answered follow-up questionnaires may have been those who were the most healthy. Second recall bias was possible because the questionnaire was cross-sectional and subjects needed to recall their medical background. However, previous studies have used the same method and established its validity [12].

Conclusions

In conclusion, our results show a strong inverse relationship between the cardiorespiratory fitness of young male athletes and the development of type 2 diabetes later in life. This relationship is independent of age, year of graduation, BMI, smoking, and college sports club participation. We conclude that cardiorespiratory fitness at a young age can predict type 2 diabetes later in life even among Japanese male athletes.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

YS carried out the interpretation of follow-up data, performed the statistical analysis and drafted the manuscript. SK participated in the design of this study and carried out the follow-up questionnaire research. YK carried out

the interpretation of the college including cardiorespiratory fitness. KA participated in the follow-up research and coordination of alumnus. HD conceived of this study and helped to draft the manuscript. All authors read and approved the final manuscript.

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INTERNAL MEDICINE

□ ORIGINAL ARTICLE □

Evaluation of Myocardial Triglyceride Accumulation Assessed on ^{1}H-Magnetic Resonance Spectroscopy in Apparently Healthy Japanese Subjects

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Abstract

Objective Proton magnetic resonance spectroscopy (^{1}H-MRS) enables the clinician to noninvasively assess the amount of ectopic fat in the liver, skeletal muscle and myocardium. Recent studies have reported that the myocardial triglyceride (TG) content is associated with aging, metabolic disorders and cardiac dysfunction. However, the clinical usefulness of myocardial TG measurements in Japanese subjects has not been fully investigated.

Methods The myocardial TG content was evaluated using ^{1}H-MRS in 37 apparently healthy Japanese subjects, and the left ventricular function was measured on cardiac magnetic resonance imaging (MRI). Blood pressure, body composition and biochemical markers were measured in a fasting state, and cardiopulmonary exercise testing (CPX) was performed to evaluate exercise capacity.

Results The mean myocardial TG content was 0.85±0.40%. The myocardial TG content was significantly associated with the percent body fat (r=0.39), serum triglyceride level (r=0.40), estimated glomerular filtration rate (r=-0.37), anaerobic threshold (r=-0.36), maximal load of CPX (r=0.39), left ventricular end-diastolic volume (r=-0.41) and left ventricular end-systolic volume (LVESV) (r=-0.51) (all: $p<0.05$). In a multivariate analysis, the LVESV was found to be an independent factor of the myocardial TG content.

Conclusion ^{1}H-MRS may be useful for assessing the associations between the myocardial TG content and various clinical parameters, including those reflecting obesity, metabolic disorders, cardiac morphology and exercise capacity, noninvasively, even in Japanese subjects.

Key words: myocardial triglyceride content, magnetic resonance spectroscopy, metabolic disorders, exercise capacity

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Introduction

Ectopic fat is associated with obesity as well as various metabolic disorders and cardiovascular diseases (1-3). In animal studies, elevated myocardial triglyceride (TG) levels trigger pathological changes, including myocardial apoptosis, left ventricular (LV) contractile dysfunction, LV diastolic dysfunction and LV remodeling (4, 5).

Proton magnetic resonance spectroscopy (^{1}H-MRS) enables the noninvasive monitoring of TG accumulation in human myocardial tissue. Previous studies have demonstrated

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that the myocardial TG content measured using ^{1}H-MRS is associated with age (6) and the presence of diabetes mellitus (7) and myocardial systolic dysfunction (4, 8, 9) or diastolic dysfunction (6, 10). In addition, progressive caloric restriction has been shown to induce a dose-dependent increase in the myocardial TG content (11), whereas endurance training reduces this parameter (12). We recently reported that the myocardial TG content is significantly lower in endurance athletes than in healthy subjects (13).

The aim of this study was to assess the associations between the myocardial TG content measured on ^{1}H-MRS and metabolic parameters, cardiac morphology, the left ventricular function and exercise capacity in apparently healthy Japanese subjects.

Materials and Methods

Subjects

A total of 37 apparently healthy Japanese men were recruited through a local advertisement. All subjects were 20-61 years of age and not currently receiving any medical treatment. Individuals with findings of acute or chronic diseases on a medical examination were excluded. The ethics committee of Juntendo University approved the study protocol and all subjects provided their written informed consent before participating in this study, according to the guidelines of the Declaration of Helsinki.

Measurements

Body composition was assessed in terms of skeletal muscle mass and body fat after overnight fasting using a multi-frequency bioelectrical impedance analysis with eight tactile electrodes (MF-BIA8; In-Body 720, Biospace, Seoul, Korea) (14). The subject's apparatus provides measurements of the fat mass, fat-free mass and percentage body fat. Each patient's personal activity level was assessed using the international physical activity questionnaire (IPAQ) (15).

Standard laboratory tests were performed under fasting conditions before the ^{1}H-MRS procedure. Serum lipid profiles were determined according to enzymatic methods, including the total cholesterol (Symex, Kobe, Japan), high-density lipoprotein (HDL) cholesterol and triglyceride (Sekisui Medical, Tokyo, Japan) levels, using a BioMajesty JCA-BM8060 analyzer (Japan Electron Optics Laboratory, Tokyo, Japan). The Friedewald formula was used to calculate the level of serum low-density lipoprotein (LDL) cholesterol. The serum insulin level was assessed using a chemiluminescent enzyme immunoassay with the Lumipulse presto II analyzer (Fujirebio, Tokyo, Japan). The homeostasis model assessment index (HOMA-IR) was calculated to estimate the degree of insulin resistance based on the fasting insulin and glucose levels, as follows: insulin (μU/mL) × glucose (mmol/L)/22.5 (16). The free fatty acid (FFA) level was measured using a standard assay (Eiken chemical, Tokyo, Japan) and BioMajesty JCA-BM2250 analyzer (Japan Electron Optics Laboratory, Tokyo, Japan). The serum N-terminal pro-brain natriuretic peptide (NT-proBNP) level was determined using an electrostatically controlled linear inchworm actuator on modular analytics (HITACHI Hi-Technologies, Tokyo, Japan). Finally, the HbA1c level was determined in whole-blood samples according to a latex-enhanced immunoassay (Fujirebio, Tokyo, Japan).

MRI and MRS

Cardiac magnetic resonance imaging (MRI) and ^{1}H-MRS were conducted on a MAGNETOM Avanto 1.5-Tesla MRI system (Siemens Medical Solution, Erlangen, Germany), as previously reported (13). In brief, each subject rested in the supine position with a belt placed around the upper part of the abdomen in order to minimize respiratory movements. LV functional and morphological parameters were determined using a specially designed software program (Argus; Siemens Medical Systems, Erlangen, Germany) (17, 18) on a separate workstation. The endocardial and epicardial LV borders were traced manually on short-axis cine images obtained at end-systole and end-diastole. The LV end-diastolic volume (EDV), LV end-systolic volume (ESV), LV ejection fraction and stroke volume were calculated using Simpson's method. Furthermore, the peak LV ejection and filling rates were automatically derived from LV volume-time curves.

In order to quantify the myocardial TG content, a 10×10×20-mm^3 voxel was positioned within the ventricular septum on cine dynamic cine-mode images (Fig. 1). The spectrum of water and lipids was acquired using point-resolved spectroscopy (PRESS) with a repetition time (TR) of at least 4,000 ms and echo time (TE) of 30 ms. The myocardial TG signals were acquired at 1.4 ppm from water suppressed spectra. The water signals were acquired at 4.7 ppm from non-water suppressed spectra (Fig. 1). The areas under the curves for the water and lipid peaks were quantified according to standard line-fitting procedures (Siemens Syngo Spectroscopy, Siemens Medical Solution). The myocardial TG levels are expressed as the lipid to water ratios (%) (13, 19-21).

Measurement of cardiopulmonary fitness

An incremental cycling test was performed on a Corival 400 (Lobe B.V., Groningen, Netherlands) with an expiratory gas analyzer (Vmax-295, SensorMedics, Yorba Linda, USA) in order to measure the anabolic threshold (AT) and maximal oxygen consumption (VO_{2max}), as previously described (22). After three minutes of rest, a three-minute warm-up test was performed at 40 W, followed by ramp loading (15-30W/min) until subjective exhaustion. According to the American Thoracic Society/American College of Chest Physicians (ATS/ACCP) guidelines, AT corresponds to the change in slope midrange of the VO_2/VCO_2 response curve (V-slope method). In cases in which the AT point was not identified on the V-slope, we used the point at which VE/VO_2 starts to increase while VE/VCO_2 remains constant, with VE corresponding to the total minute ventilation (23).

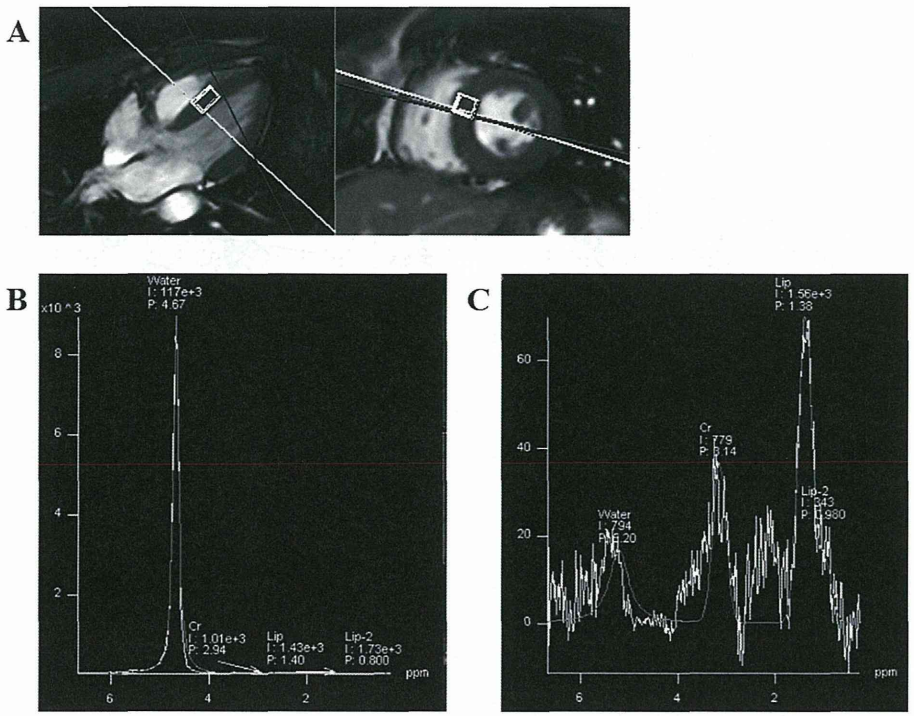

Figure 1. **Representative results of the ^{1}H-MRS spectra in a healthy subject. A: Myocardial voxel localization for ^{1}H-MRS in four-chamber and short-axis views. B: ^{1}H-MR spectra without water suppression. C: ^{1}H-MR spectra without water suppression.**

Table 1. **Clinical Characteristics**

	Total (n = 37)
Age, years	30.6 ± 8.1
Body height, m	1.728 ± 0.051
Body weight, kg	68.0 ± 6.5
Body mass index, kg/m^2	22.7 ± 2.0
Skeletal muscle mass, kg	32.9 ± 8.3
Body fat weight, kg	13.0 ± 4.4
Percent of body fat, %	18.5 ± 5.4
Neck circumference, cm	37.0 ± 2.3
Waist circumference, cm	80.0 ± 5.7
Total cholesterol, mg/dL	183 ± 29
Triglyceride, mg/dL	102 ± 73
LDL–cholesterol, mg/dL	108 ± 29
HDL–cholesterol, mg/dL	55 ± 11
Fasting free fatty acid, μEq/L	326 ± 161
Fasting blood glucose, mg/dL	92 ± 8
Insulin, μU/mL	5.9 ± 3.7
HOMA–IR	1.3 ± 0.9
HbA1c, % (NGSP)	4.7 ± 0.2
Creatinine, mg/dL	0.83 ± 0.09
eGFR, mL/min/m^2	91.6 ± 12.4
NT-proBNP, ng/L	15.1 ± 13.3
Urinary acid, mg/L	6.0 ± 0.9
Anaerobic threshold, mL/kg/min	19.0 ± 5.2
VO_2max, mL/kg/min	43.2 ± 8.0
CAVI	6.5 ± 0.7
IPAQ score	2,318 ± 1,605

Values are mean ± SD. 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, VO_2max: maximal oxygen intake, CAVI: cardio ankle vascular index, IPAQ: international physical activity questionnaire

Table 2. **MRI Variables**

	Total (n = 37)
LV ejection fraction, %	50.2 ± 5.4
LV end diastolic volume, mL	157 ± 26
LV end systolic volume, mL	77 ± 16
Stroke volume, mL	80 ± 14
Cardiac output, L/min/m^2	4.9 ± 1.0
LV myocardial mass, g	124 ± 17
Peak ejection rate, mL/sec	608 ± 234
Peak filling rate, mL/sec	672 ± 251

Values are mean ± SD. LV: left ventricular

Evaluation of atherosclerotic parameters

The parameter of atherosclerosis, the cardio-ankle vascular index (CAVI), was calculated automatically using the VaSera VS-1500 AN (Fukuda Denshi, Tokyo, Japan) (24, 25).

Statistical analysis

The values are expressed as the mean ± standard deviation (SD). Variables not exhibiting a normal distribution were transformed into natural logarithmic values prior to the statistical analyses. Pearson's correlation coefficients were calculated between the myocardial TG content and the other parameters. In order to assess determinants of the myocardial TG content, we performed univariate and multivariable linear regression analyses. All statistical analyses were conducted using the SPSS version 20 software package (SPSS, Chicago, USA). A p value of less than 0.05 was considered to be statistically significant.

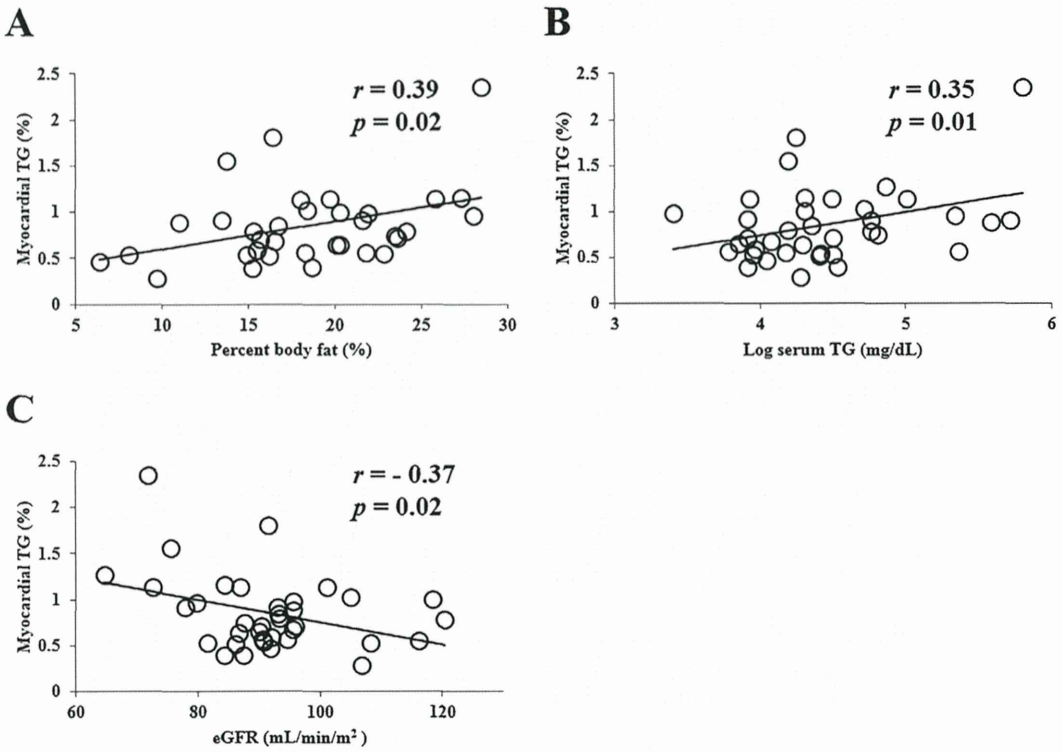

Figure 2. **Correlations between the myocardial TG content and the clinical variables. A: Correlation between the myocardial TG content and percent body fat. B: Correlation between the myocardial TG content and the serum TG level with logarithmic transformation. C: Correlation between the myocardial TG content and estimated glomerular filtration rate.**

Results

The clinical characteristics of the study subjects are summarized in Table 1. The mean age was 30.5±8.1 years, the mean body mass index was 22.7±2.0 kg/m^2 and the mean maximum VO_2 was 43.2±8.0 mL/kg/min. None of the subjects were obese or had abnormal parameters on the blood analyses.

The MRI and MRS variables are shown in Table 2. None of the subjects had an abnormal ejection fraction, cardiac mass volume, peak ejection fraction or filling rate. The myocardial TG content was positively correlated with the percent body fat (r=0.39, p=0.02) and serum TG level (r= 0.35, p=0.001) (Fig. 2) and negatively correlated with the estimated glomerular filtration rate (eGFR; r=-0.37, p=0.02), AT (r=-0.36, p=0.02), maximal load of cardiopulmonary exercise testing (CPX) (r=-0.40, p=0.01), left ventricular end-diastolic volume (LVEDV; r=-0.42, p=0.01) and left ventricular end-systolic volume (LVESV; r=-0.51, p=0.01) (Fig. 3). No significant correlations were noted between the myocardial TG content and the CAVI (r=0.16, p=0.37) or and the IPAQ score (r=-0.25, p=0.14).

The AT level strongly correlated with the maximal CPX load (r=0.81, p<0.0001). The LVESV level also correlated with the LVEDV (r=0.82, p<0.0001). Moreover, the myocardial TG content and serum TG level were associated with insulin resistance and obesity, and the relationship between the myocardial TG content and the eGFR was affected by aging. Therefore, we performed a multivariate analysis including age, BMI, serum TG, eGFR and LVESV. In this model, the LVESV was an independent factor of the myocardial TG content (Table 3).

Discussion

The present study demonstrated significant associations between the myocardial TG content and the percent body fat, serum TG level, eGFR, anaerobic threshold, maximal load of CPX, LVEDV and LVESV in apparently healthy Japanese subjects. In addition, the LVESV value was found to be an independent factor of the myocardial TG content.

The generation of myocardial TG is closely dependent on myocardial lipid metabolism. The accumulation of myocardial TG is primarily determined by the supply of the fatty acids and mitochondrial energy-producing efficiency (26). Moreover, myocardial lipid metabolism is regulated by a complex equilibrium between the supply of fatty acids to the heart, competing energy substrates, energy demand and supply of oxygen to the heart, uptake and esterification of fatty acids and control of the mitochondrial function (i.e. fatty acid oxidation and electron transport chain activity) (26). Therefore, it is important to assess the myocardial TG content using ^{1}H-MRS noninvasively in the clinical setting.

Previous studies have reported that the myocardial TG

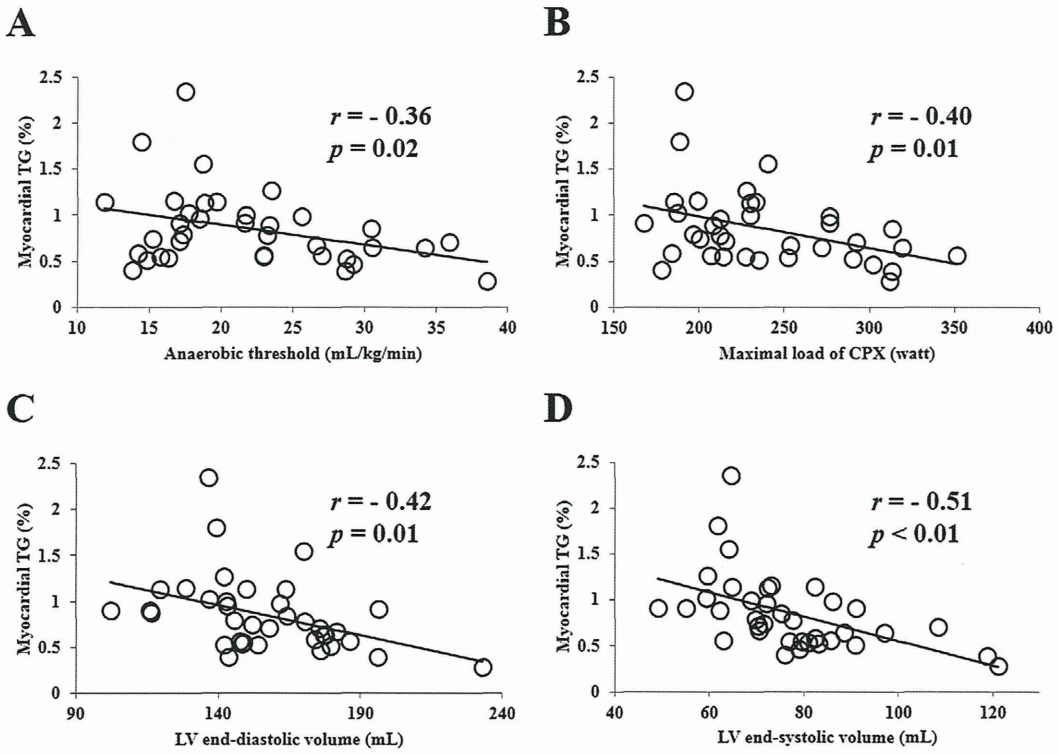

Figure 3. **Correlations between the myocardial TG content and the MRI parameters. A: Correlation between the myocardial TG content and anaerobic threshold. B: Correlation between the myocardial TG content and maximal load of cardiopulmonary exercise. C: Correlation between the myocardial TG content and left ventricular (LV) end-diastolic volume. D: Correlation between the myocardial TG content and LV end-systolic volume.**

Table 3. **Multivariable Linear Regression Analyses**

Factors	ß	p value
Age	-0.0036	0.69
BMI	0.036	0.28
Serum TG	0.00099	0.22
eGFR	-0.0070	0.22
LVESV	-0.011	0.021

BMI: body mass index, TG: triglyceride, eGFR: estimated glomerular filtration rate, LVESV: left ventricular end-systolic volume

content is related to obesity, metabolic disorders and cardiac dysfunction (1, 2, 4, 5, 7, 27). The present study confirmed an association between the myocardial TG content and parameters of metabolic disorders, including the percent body fat and serum TG levels, even in apparently healthy subjects. These associations are supported by the well-established notion that increased body fat results in insulin resistance and high TG levels. In addition, a previous study suggested that the myocardial TG content is associated with obesity via a positive association with the plasma FFA concentration (8). However, we found no correlations between the FFA level and myocardial TG content in our healthy subjects (data not shown). The present study subjects did not include obese patients or those with impaired glucose tolerance with a decreased mitochondrial function. In addition, we enrolled several healthy subjects with exercise habits. Endurance exercise regulates lipoprotein lipase synthesis and the mitochondrial function, primarily β-oxidation (28). The background factors of the study subjects may therefore account for differences in results between the present study and previous reports.

In the current analysis, we found that morphological cardiac parameters were negatively correlated with the myocardial TG content, including the LVEDV and LVESV. In previous reports, the myocardial TG content has been shown to positively correlate with LV mass weight per LV volume according to the degree of LV hypertrophy (4). Furthermore, we recently reported that athletes exhibit lower myocardial TG levels (13). We also included several endurance athletes in the present study; however, similar trends were obtained after excluding these subjects (data not shown). Moreover, the LVESV level was found to be an independent predictor of the myocardial TG content. These data suggest that measuring the myocardial TG content is useful for assessing the relationship between cardiac remodeling and cardiac lipid metabolism, although further investigations are needed to clarify the precise mechanisms.

Finally, our data showed a negative correlation between the myocardial TG content and AT. A previous study demonstrated a negative relationship between the myocardial TG content and cardiopulmonary fitness in obese women (27). Reduced cardiorespiratory fitness predisposes to individuals to cardiovascular disease and predicts premature death (29, 30), and the level of cardiorespiratory fitness de-

pends on both the degree of obesity and age. Our next study will investigate the impact of clinical interventions on the myocardial TG content and exercise tolerance.

The present study is associated with several limitations. First, this was a single-center study with a small sample size. Second, we included only male subjects. Finally, we did not perform oral glucose tolerance tests. Therefore, some subjects with impaired glucose tolerance may have been included in our study population; however, no obese individuals or patients with impaired glucose tolerance with an obviously decreased mitochondrial function were assessed.

Conclusion

^{1}H-MRS may be useful for noninvasively assessing the associations between the myocardial TG content and various clinical parameters, including those indicative of obesity, metabolic disorders, cardiac morphology and exercise capacity, even in healthy Japanese subjects.

The authors state that they have no Conflict of Interest (COI).

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