

Research article

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## Required muscle mass for preventing lifestyle-related diseases in Japanese women

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### Abstract

**Background:** Since it is essential to maintain a high level of cardiorespiratory fitness to prevent life-style related disease, the Ministry of Health, Labour and Welfare of Japan in 2006 proposed to determine the maximal oxygen uptake ( $Vo_2max$ :  $mL \cdot kg^{-1} \cdot min^{-1}$ ) reference values to prevent life-style related diseases (LSRD). Since muscle mass is one of the determinant factors of  $Vo_2max$ , it could be used as the reference parameter for preventing LSRD. The aim of this study was to determine and quantify the muscle mass required to maintain the  $Vo_2max$  reference values in Japanese women.

**Methods:** A total of 403 Japanese women aged 20–69 years were randomly allocated to either a validation or a cross-validation group. In the validation group, a multiple regression equation, which used a set of age and the percentage of muscle mass (%MM, percentage of appendicular lean soft tissue mass to body weight), as independent variables, was derived to estimate the  $Vo_2max$ . After the equation was cross-validated, data from the two groups were pooled together to establish the final equation. The required %MM for each subject was recalculated by substituting the  $Vo_2max$  reference values and her age in the final equation.

**Results:** The mean value of required %MM was identified as ( $28.5 \pm 0.35\%$ ). Thus, the present study proposed the required muscle mass (28.5% per body weight) in Japanese women to maintain the  $Vo_2max$  reference values determined by the Japanese Ministry of Health Labour and Welfare.

**Conclusion:** The estimated required %MM (28.5% per body weight) can be used as one of the reference parameters of fitness level in Japanese women.

## Background

Previous epidemiologic and clinical evidence indicate that a poor cardiorespiratory fitness is a major risk factor for life-style related diseases (LSRD) such as obesity, hypertension, hypercholesterolaemia, arteriosclerosis and diabetes [1-4]. Moreover, low cardiorespiratory fitness has been found to be a predictor of cardiovascular disease (CVD) mortality, and all-cause mortality [5-8]. Thus, it is essential to maintain a high level of cardiorespiratory fitness to prevent LSRD.

Cardiovascular fitness is usually evaluated as the maximal oxygen uptake per body mass ( $Vo_{2max}$ ,  $mL \cdot kg^{-1} \cdot min^{-1}$ ). The Japanese Ministry of Health Labour and Welfare in 2006 proposed  $Vo_{2max}$  reference values for each age group to prevent LSRD [9]. These  $Vo_{2max}$  reference values were determined by the "Committee for the Determination of the Recommended Exercise Allowance and Exercise Guide" established in August 2005, and were referenced in the "Exercise and Physical Activity Reference Quantity for Health Promotion 2006 (EPAR2006)". Originally, the "Recommended Quantity of Exercise for Health Promotion (1989)" had been formulated to mainly target the prevention of coronary artery disease. With the passage of more than 15 years following the establishment of this standard, the morbidity pattern of people has worsened and LSRD have increased in prevalence. In order to face this situation, the EPAR2006 was made based on the latest scientific evidence, and was designed to maintain and promote the health of people and prevent LSRD by improving their capacity for physical activity and exercise. These  $Vo_{2max}$  reference values proposed in the EPAR2006 were determined by experts through the systematic review of literature regarding the relationship between  $Vo_{2max}$  and LSRD such as obesity, hypertension, hypercholesterolemia, diabetes, cerebrovascular disease, CVD mortality and all-cause mortality.

It is well known that  $Vo_{2max}$  decreases with age [10-20]. It has been suggested that the age-related decline in  $Vo_{2max}$  is a consequence of attenuation of central and peripheral functions such as stroke volume, heart rate max ( $HR_{max}$ ), peripheral  $O_2$  extraction, and lean body mass (LBM) or muscle mass [19,21-25]. Among these determinants, reductions in  $HR_{max}$  and LBM or muscle mass have been suggested to be primary factors [26,27]. While many studies on cardiovascular fitness have focused on cardiac measurements, it should be emphasized that muscle mass is one of the critical determinants of  $Vo_{2max}$  [13,14,19,24,26,28-30] since the amount of tissue available to extract oxygen during maximal exercise, i.e., muscle, can directly contribute to the value of  $Vo_{2max}$ . For example, Sanada et al. reported the MRI-measured lower body skeletal muscle mass was closely associated to the absolute  $Vo_{2max}$  during running [28,30]. Additionally, the

age-related decrement in  $Vo_{2max}$  can be related to the age-associated muscle loss [24,19]. Further, it is important to notice that LBM or muscle mass can be maintained to some degree by exercise training, while such training cannot prevent age-related declines in  $HR_{max}$  [26,27].

Therefore, we hypothesized that a certain level of muscle mass required to maintain sufficient cardiovascular fitness is present and that it could be a limiting factor of age-related  $Vo_{2max}$  attenuation. Based on this hypothesis, it is advantageous to Japanese women's health to propose such muscle mass required to maintain sufficient  $Vo_{2max}$ . Thus, the purpose of this study was to determine a required value of muscle mass to maintain the  $Vo_{2max}$  reference value determined by the Japanese Ministry of Health Labour and Welfare in 2006 (Ministry of Health, Labour and Welfare of Japan 2006).

## Methods

### Subjects

A group of 403 Japanese women aged 20 to 69 years were randomly allocated to either a validation group (V-group,  $n = 201$ ) or a cross-validation group (CV-group,  $n = 202$ ). The subjects were recruited from the community around the National Institute of Health and Nutrition. All subjects were active and free of overt CVD assessed using a medical history questionnaire. All assessments were conducted at the National Institute of Health and Nutrition between February 2004 and October 2006. The study was approved by the Ethics Committee of the National Institute of Health and Nutrition, and written consent was obtained from all participants.

### Percentage of muscle mass

The lean soft tissue mass of legs and arms were measured with a whole-body Dual Energy X-ray Absorptiometry (DXA) scanner (Hologic QDR-4500, Hologic INC., Waltham, MA, USA). The body regions were delineated according to specific anatomical landmarks using manual DXA analysis software (version 11.2.3). The appendicular lean soft tissue mass was calculated as a sum of the lean soft tissue mass of the legs and the arms. The lean soft tissue mass of extremities assessed using DXA was assumed to represent appendicular skeletal muscle mass along with a small and relatively constant amount of skin and underlying connective tissues. The percentage of muscle mass (%MM) was calculated as follows;

$$\%MM (\%) = (\text{Appendicular lean soft tissue mass}) / \text{Body weight} \times 100.$$

### $Vo_{2max}$

We assessed peak oxygen uptake ( $Vo_{2peak}$ ;  $mL \cdot kg^{-1} \cdot min^{-1}$ ) instead of  $Vo_{2max}$  as an index of cardiorespiratory fitness, which is defined as the highest level of oxygen uptake that

is determined by the protocol of a graded exercise load. The  $Vo_{2peak}$  was measured using the incremental cycle exercise. An initial work intensity of 30 W or 60 W was selected for each patient based on the patient's fitness level. The work intensity was increased thereafter by a step of 15 W/min, until the subject was not able to maintain the required pedaling frequency of 60 rpm. The heart rate and rating of perceived exertion (RPE) were monitored throughout the exercise. The  $O_2$  consumption and the minute ventilation were monitored during each 1-min exercise stage (two 30 sec samplings for each stage), after RPE reached 18. The expired air was collected using Douglas bags. Expired  $O_2$  and  $CO_2$  gas concentrations were measured using a mass spectrometer (ARCO-1000A, ARCO SYSTEM, Chiba, Japan), and gas volume was measured using a dry gas meter (DC-5C Shinagawa Seiki, Tokyo, Japan). If the subject became exhausted and was not able to keep the pedaling frequency at 60 rpm, it was decided that the maximum effort had been achieved and the test was terminated. The highest value of  $Vo_2$  during the exercise test was designated as  $Vo_{2peak}$ . Note that the oxygen uptake obtained in this procedure is referred to as  $Vo_{2peak}$  to discriminate this from  $Vo_{2max}$  in the strict definition. However, we equate the obtained  $Vo_{2peak}$  to  $Vo_{2max}$  in the present study since the  $Vo_{2max}$  reference value was determined using both  $Vo_{2max}$  and  $Vo_{2peak}$  as mentioned in the next section.

**$Vo_{2max}$  reference values**

The Japanese Ministry of Health Labour and Welfare proposed  $Vo_{2max}$  reference values to prevent life-style related illness for women [9]. The  $Vo_{2max}$  reference values are provided for each age group. The procedure to determine  $Vo_{2max}$  reference values was described in the EPARQ2006 [9]. In brief, these  $Vo_{2max}$  reference values were determined by experts through a systematic review of literature. The target age was 6 years and older. The target LSRD were obesity, hypertension, hyperlipemia, diabetes mellitus, cerebrovascular disorders, death due to circulatory diseases, osteoporosis, ADL and total mortality. By means of this systematic review, the threshold values of the  $Vo_{2max}$  or  $Vo_{2peak}$  at which the morbidity of LSRD statistically increases in each age group were collected from the literature. The average values of these threshold values for each age group were then calculated and designated as the  $Vo_{2max}$  reference values for preventing LSRD. The identified  $Vo_{2max}$  reference values ( $mL \cdot kg^{-1} \cdot min^{-1}$ ) were 33 (20–29 yr), 32 (30–39 yr), 31 (40–49 yr), 29 (50–59 yr), and 28 (60–69 yr).

**Analyses**

First, a single regression analysis was used to test the correlation between age and  $Vo_{2max}$ , and between %MM and  $Vo_{2max}$  in V-group. Then, a multiple regression analysis was performed using  $Vo_{2max}$  as a dependent variable,

and age and %MM as the independent variables. This analysis was based on the hypothesis that  $Vo_{2max}$  can be accounted for by age and %MM. In this hypothesis, we assumed that the age factor included  $Vo_{2max}$  determinant factors related to aging except for muscle mass, such as  $HR_{max}$ , maximal stroke volume, and peripheral  $O_2$  extraction [21-23,25,27]. The validity of the prediction by the obtained regression equation was tested by applying the obtained regression equation to the CV-group. After the equation was cross-validated, the data from the two groups were pooled together to obtain the final prediction equation and in the subsequent analysis.

The purpose of the final prediction equation was to obtain the required %MM to maintain the reference  $Vo_{2max}$  value in each age group. Thus, the required %MM for each subject was recalculated by assigning the  $Vo_{2max}$  reference values and age in the final prediction equation. If the difference of the required %MM among the age groups was very small, the mean value of the required %MM was calculated to be used in the following analysis. To test the validity of the required %MM, the correlation between the sufficiency of  $Vo_{2max}$ , i.e., individual's  $Vo_{2max}$  as the percentage of the  $Vo_{2max}$  reference values (%  $Vo_{2max}$  reference values), and the sufficiency of the required %MM, i.e., individual's %MM as the percentage of the required %MM (%required-%MM), were tested.

All data are reported as means ± standard deviations (SD).  $P < 0.05$  was used as a level of significance for all comparisons.

**Results**

**Physiological characteristics**

The physiological characteristics for each group are shown in Table 1. There were no significant physiological differences between V-group and CV-group.

**Relationship between age and  $Vo_{2max}$  in V-group**

$Vo_{2max}$  in V-group was from 16.4 to 56.9  $ml \cdot kg^{-1} \cdot min^{-1}$  (mean  $33.5 \pm 7.9$ ) (Table 1). As expected, a strong nega-

**Table 1: Characteristics of validation and cross-validation group**

	V-group	CV-group
n	202	201
Age (yr)	41.4 ± 16.7	41.6 ± 16.9
Height (cm)	158.5 ± 6.4	157.9 ± 6.1
Body weight (kg)	54.4 ± 7.4	53.9 ± 7.3
Body mass index ( $kg/m^2$ )	21.6 ± 2.7	21.7 ± 2.9
Appendicular muscle mass (kg)	16.4 ± 2.4	16.1 ± 2.3
% MMI (%)	30.3 ± 3.2	30.0 ± 3.4
$Vo_{2max}$ ( $ml \cdot kg^{-1} \cdot min^{-1}$ )	33.5 ± 7.9	32.7 ± 7.7

mean ± SD, V-group, Validation group; CV-group, Cross-validation group; %MM, percentage of muscle mass

tive linear correlation was found between  $VO_2\text{max}$  and age (Figure 1). The decrement was  $2.58 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$  per decade. The  $VO_2\text{max}$  reference values for each age group in the EPAR2006 were superimposed in Figure 1. With increasing age, the proportion of subjects with  $VO_2\text{max}$  values below the reference  $VO_2\text{max}$  values increased.

**Relationship between  $VO_2\text{max}$  and %MM in V-group**

%MM in V-group was from 18.7 to 37.3% (mean  $30.3 \pm 3.2\%$ ) (Table 1). There was also a strong correlation between  $VO_2\text{max}$  and %MM, while the correlation was positive (Figure 2).

**Multiple-regression analysis in V-group**

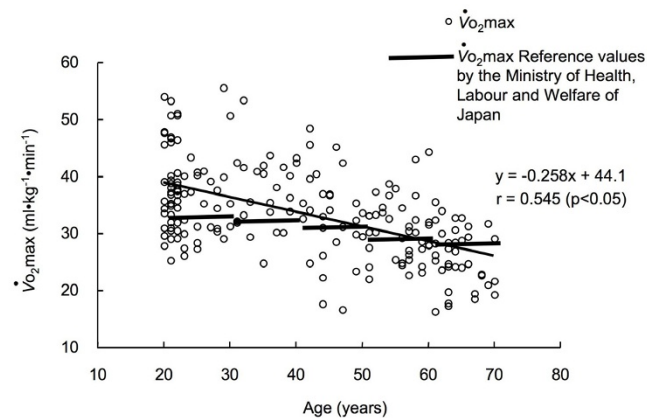
Multiple regression analysis in V-group revealed that age ( $R^2 = 0.286$ ) and %MM ( $R^2 = 0.540$ ) were significant ( $p < 0.0001$ ) contributors to the prediction of the measured  $VO_2\text{max}$ . The multiple regression equation obtained in the V-group was the following:  $VO_2\text{max} = -0.135 \times \text{Age} + 1.315 \times \%MM - 0.799$ . In this equation,  $R^2$  and SEE were 0.522 and  $5.4 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ , respectively.

**Cross-validation of the multiple regression equation**

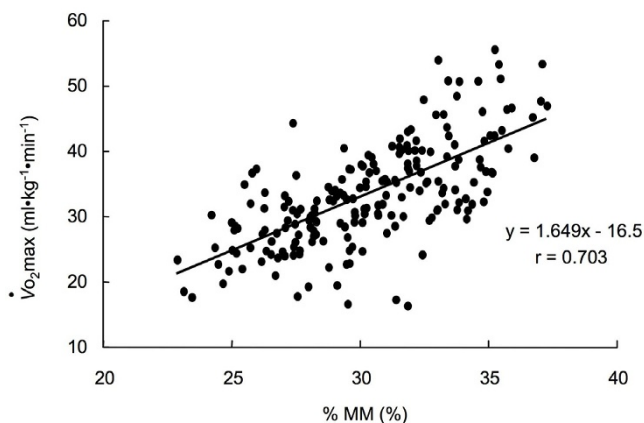
The multiple regression equation derived from the V-group was used to predict  $VO_2\text{max}$  in the CV-group. Figure 3 shows the residual plot. There was not statistically significant correlation between the predicted  $VO_2\text{max}$  and residual error ( $p > 0.05$ ). Thus, the residual plot indicates that there was no bias in the prediction of  $VO_2\text{max}$  of the CV-group using the multiple regression obtained in the V-group.

**Final prediction equation**

Data from the two groups were pooled to generate the final equations:



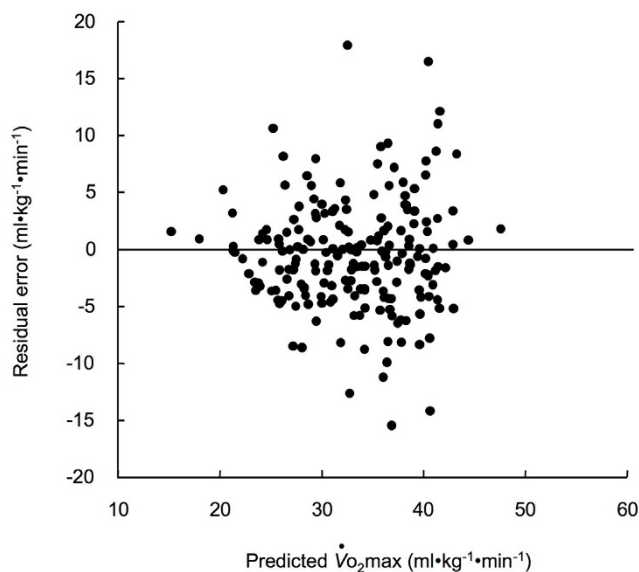
**Figure 1**  
The relationship between age and  $VO_2\text{max}$  in the V-group. The  $VO_2\text{max}$  reference values by the Japanese Ministry of Health Labour and Welfare were shown for reference.



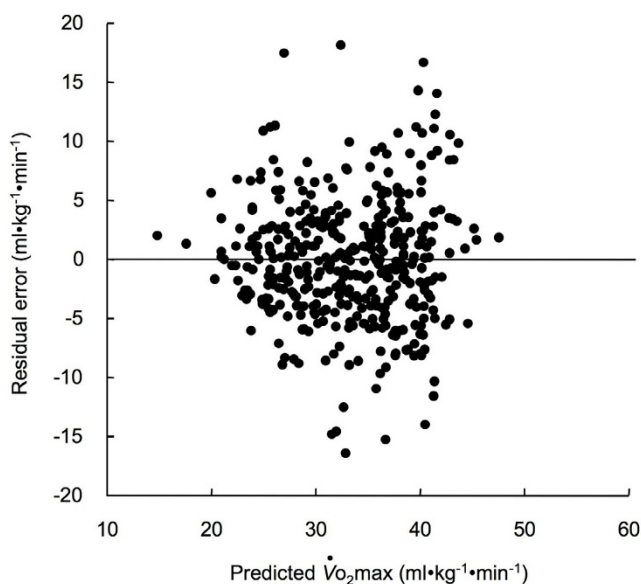
**Figure 2**  
Relationship between percentage of muscle mass (%MM) and  $VO_2\text{max}$  in the V-group.

$$VO_2\text{max} = -0.131 \times \text{Age} + 1.344 \times \%MM - 2.035. \quad (1)$$

In the final equation, analysis revealed that age ( $R^2 = 0.282$ ) and %MM ( $R^2 = 0.570$ ) were significant ( $p < 0.0001$ ) independent contributors to the prediction of the measured  $VO_2\text{max}$ . Figure 4 shows the residual plot of the multiple-regression. There was no statistically significant correlation between the predicted  $VO_2\text{max}$  and residual error ( $p > 0.05$ ). Thus, the residual plot indicates that there was no bias in the prediction of  $VO_2\text{max}$ .



**Figure 3**  
Relationship between estimated  $VO_2\text{max}$  by the multiple regression equation and the residuals for the CV-group.



**Figure 4**  
Relationship between estimated  $Vo_2max$  by the multiple regression equation and the residuals for both the V-group and the CV-group.

**Estimation of the required %MM**

The equation (1) was rearranged to predict required %MM as follow;

$$\%MM = (0.131 \times \text{Age} + 2.035 + Vo_2max)/1.344. \tag{2}$$

The required %MM was calculated by assigning the  $Vo_2max$  reference values, and age in the equation (2). The calculated required %MM was shown in Table 2. The mean value and standard deviation of required %MM was  $28.5 \pm 0.35\%$ . Figure 5 shows the relationship between the measured %MM and age with the required %MM superimposed on the plot. The older people tended to have a %MM lower than the required. With increasing age, the proportion of subjects with %MM below the required %MM increased.

**The validity of the required %MM**

Figure 6 shows the relationship between % $Vo_2max$  reference values and %required-%MM. The % $Vo_2max$  refer-

ence values positively correlated with %required-%MM ( $r = 0.651, p < 0.05$ ).

**Discussion**

The primary finding of the present study is that appendicular muscle mass of 28.5% of body weight is needed to maintain the  $Vo_2max$  reference values determined by the Japanese Ministry of Health Labour and Welfare in Japanese women. By use of the multiple-regression analysis, the regression equation of  $Vo_2max$  from age and %MM was obtained in the V-group at first. Then the validity of the regression equation was confirmed in the CV-group (Figure 3). The required %MM to maintain the  $Vo_2max$  reference values was obtained using the final regression equation using the data of V- and CV-groups (equation (2)) and the  $Vo_2max$  reference values for each age group (Table 2). There was strong correlation between percentages of the required %MM and  $Vo_2max$  reference values (Figure 6).

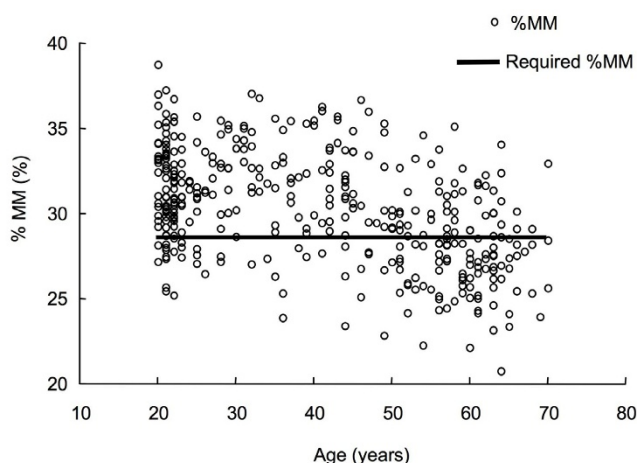
**Required muscle mass**

We propose the required %MM in Japanese women as a reference value of muscle mass for the usage of maintaining the reference value of  $Vo_2max$  proposed by the Ministry of Health Labour and Welfare of Japan. Interestingly, the calculated required %MM was not different among age groups (Table 2). Thus, we proposed the averaged required muscle mass (28.5%) as the general value for all age groups. A large portion of the subjects (68%) satisfied the required muscle mass, while with increasing age, the proportion of subjects with %MM below the required %MM increased (Figure 5). This tendency was similar to  $Vo_2max$ , i.e., with increasing age, the proportion of subjects with  $Vo_2max$  values below the reference  $Vo_2max$  values increased (Figure 1). Additionally, there was strong positive relation between percentages of  $Vo_2max$  reference values and required %MM (Figure 6). The results indicate that subjects with total muscle mass lower than 100% of the required %MM also tended to have lower  $Vo_2max$  when compared to levels of  $Vo_2max$  reference values. Thus, our result suggests that one of the reasons for insufficient  $Vo_2max$  may be insufficient %MM. Women who have %MM less than the required %MM are encouraged to increase their %MM above the required %MM to achieve the  $Vo_2max$  reference values. The required %MM can be used as an additional parameter for preventing LSRD together with the  $Vo_2max$  reference values. The

**Table 2: Required %MM for  $Vo_2max$  reference values of each age group**

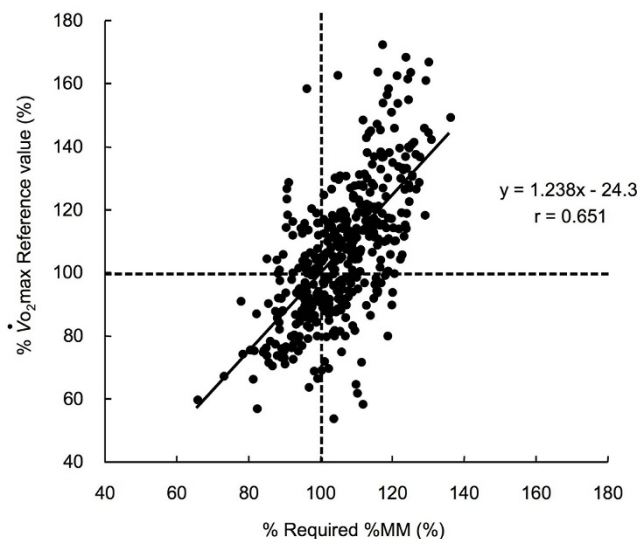
Age group	20 Y	30 Y	40 Y	50 Y	60 Y	Total
n	143	48	55	73	84	403
Required MMI (%)	$28.3 \pm 0.26$	$28.6 \pm 0.29$	$28.9 \pm 0.27$	$28.4 \pm 0.25$	$28.6 \pm 0.30$	$28.5 \pm 0.35$

Mean  $\pm$  SD; %MMI, percentage of muscle mass



**Figure 5**  
**The relationship between age and the percentage of muscle mass (%MM) in the V-group and the CV-group.** Required %MM is shown for reference.

required %MM obtained in this study is practical and appropriate for most Japanese women, because it is slightly less than the average %MM of the total number of subjects. Thus, the value is an achievable goal for most of Japanese women. Although strength training is not typically included in exercise programs targeting prevention



**Figure 6**  
**The relationship between the sufficiency of Vo<sub>2</sub>max (%Vo<sub>2</sub>max reference values) and the sufficiency of the required %MM (% required %MM) in both the V-group and the CV-group.** Solid line: regression line, dashed line: lines of 100% of Required %MM and 100% of Vo<sub>2</sub>max reference values.

of the age-related decline in Vo<sub>2</sub>max or to increase Vo<sub>2</sub>max, it would be advisable to recommend some form of strength training as well as aerobic training especially for individuals who do not achieve the required %MM.

Several prior studies demonstrated the significance of fat free mass, muscle mass, and/or muscle function to morbidity and mortality, although there are few researches targeting women [31-33]. The Japanese Ministry of Health Labour and Welfare also has admitted the importance of muscle mass and muscle function to prevent LSRD and/or mortality in EPAR2006. However practical target values have not been offered in the statement due to the lack of evidences compared to Vo<sub>2</sub>max. In this present study we determined the target value of muscle mass through the Vo<sub>2</sub>max reference values, which already has strong evidences. Although we have not confirmed the direct relation between muscle mass and LSRD morbidity and/or mortality, we believe Japanese women could aim to achieve the required %MM as one of targets for their health. Whether an increase of skeletal muscle mass would result in an improvement of exercise capacity and or reduce morbidity and mortality needs to be confirmed by future studies.

It should note that some individuals may have a large muscle mass, yet be at a high mortality risk. For example, it is well known that central obesity is one of risk factor of LSRD morbidity. Thus, it is important to remember that muscle mass is not the only important parameter but also, other risk factor should be monitored and considered together.

**Prediction of Vo<sub>2</sub>max from age and muscle mass**

The residuals of the multiple regression might be due to the approximation that all age-related determinant factors were included in age in the multiple regression. In the present model, we hypothesized that determinants such as HR<sub>max</sub>, maximal stroke volume, and peripheral O<sub>2</sub> extraction were age-related, and therefore their effects were included in the factor of age. It was suggested that HR<sub>max</sub> [14,22,26,29,34-39] and peripheral O<sub>2</sub> extraction [21,34] do decline with age, and are not influenced by exercise training. However, although maximum stroke volume was also suggested to decline with age in sedentary individuals [23], it was suggested that age-related decline of maximum stroke volume was prevented by exercise [21,34]. Thus, the simplification must be the error factor, and it is likely in future to improve the multiple regression equations using these age-related Vo<sub>2</sub>max determinants, and to improve the estimation of the required MMI.

We studied only a statistical relationship between Vo<sub>2</sub>max and muscle mass. Therefore, the results do not necessarily

suggest a cause-effect relationship. It is possible that muscle mass and  $Vo_2max$  are physiologically unrelated but indirectly correlated, i.e., people with a high  $Vo_2max$  may be more physically active and perform activities that increase muscle mass. However, muscle mass is highly likely physiologically important determinant of  $Vo_2max$  because the amount of tissue available to extract oxygen during maximal exercise directly contribute to the value of  $Vo_2max$ .

### Study limitations

The current study has limitations that require caution when interpreting and generalizing the findings reported herein. This study included only the cross-sectional design, and it did not investigate the relationship between the required %MM and the morbidity of LSRD or mortality by using a prospective design. Thus, it has not been clarified how the required %MM reflects these risks in this present study. Further investigation is required to validate the required %MM through a prospective study with the morbidity and/or mortality as an endpoint. Additionally, the potential difference between methods using %MM or absolute muscle mass (kg) as the indicator of health should be also investigated. Another limitation of this study is the results of this study are applicable to only Japanese women. The decided %MM in this study may not be able to be applicable to men and/or other racial group since they may have different characteristics of the relationship between muscle mass and  $Vo_2max$ .

### Conclusion

In conclusion, the present study proposed the required muscle mass (28.5% per body weight) in Japanese women to maintain the  $Vo_2max$  reference values determined by the Japanese Ministry of Health Labour and Welfare. This required muscle mass can be used as one of the reference parameters of fitness level in Japanese women.

### Competing interests

The authors declare that they have no competing interests.

### Authors' contributions

MM performed analysis and data interpretation as well as drafted and revised the manuscript. KM participated in the conception of this study, interpretation of the analysis and critically reviewed this manuscript, and provided comment as Statistical expertise. HK, YG, KY, MT, TO, CU and SK performed data analysis and interpretation, and provided comment and review of the manuscript. MH and IT designed the project, assisted with data interpretation and provided comment and revisions for the manuscript. MM designed the project, participated in the conception of this study, interpretation of the analysis and critically reviewed this manuscript. All authors read

and give final approval of the final manuscript for publication.

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