Measurement of net muscle volume in patients with muscular dystrophy using muscle CT for prospective muscle volume analysis

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TITLE: Measurement of net muscle volume in patients with muscular dystrophy using muscle CT for prospective muscle volume analysis

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muscular dystrophy, muscle volumetry, outcome measure, CT, DXA

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Figures: 6, Table: 1

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Abstract

Objective:

Muscle volume in patients with muscle disease is an index of disease progression. The aim of this study is to show a new method of muscle volumetry using CT of thigh muscles.

Subjects

1. For muscle volumetry using CT and DXA, 13 patients with muscle disease participated.
2. For prospective CT volumetry, 12 patients participated for 4 years.

Methods:

1.a. Helical CT scanner imaging of the thigh was performed. CT images were analyzed applying estimated functions, and the accumulation of outcomes resulted in muscle volumes.

1.b. Pencil beam DXA was used in these patients and the muscle mass of the thigh was calculated by the attached software.

2. To prospectively compare muscle volumes, CT images of the mid-thigh were measured. We referred to this method as net muscle volumetry.

Results:

1.a. Muscle volumes of the thigh on one side were calculated as between 300 and 3400 cm^3 by CT.

1.b. Muscle masses of the thigh calculated from DXA were estimated at between 1100 and 5000 g. Results closely corresponded to muscle volumes calculated by CT with an interclass correlation coefficient of 0.993.

2. Thigh net muscle volumes of 7 patients who complained of gait disturbance decreased in four years ($p<0.01$).

Conclusions:

Measurement of net muscle volume using CT, which was validated by the muscle mass calculated from DXA, was developed. Net muscle volume decrements during four years supported the reliability of this method. This less arbitrary method is suitable for assessment of muscle volume in patients with muscular dystrophy.

(250 words)

Article Summary

Article focus

- ✓ Muscle volume in patients with muscle disease is an index of disease progression and the efficacy of therapy, but muscle volumetry in which the muscle volume decrease could be shown in the patients with muscular dystrophies during several years has not been conducted so far.
- ✓ There were some reports which showed estimated muscle volume were correlated to muscle power or functional rating scale in patients with muscular dystrophy using CT, MRI and DXA(dual energy X-ray absorptiometry).
- ✓ We proposed a method of muscle volumetry using our own estimating function to muscle CT images in patients with muscular diseases. And we demonstrated the long-term applicable method by calculating constant zones. And we called our method as net muscle volumetry.

Key messages

- ✓ The muscle volume using our method are closely correlated to the muscle mass using DXA, and the decrement of muscle volume are corresponded to the aggravation of muscle functional classification. These findings validated our method.
- ✓ We demonstrated the net muscle volume decrease or consistency during four years, and that also supported the reliability of this method.
- ✓ In this study, muscle was mostly automatically differentiated with the estimating function applying CT values of image pixels, except for manual elimination of skin or vessels. . This less arbitrary method is suitable for assessment of muscle volume in patients with muscular dystrophy under clinical treatment trials and will become a strong clinical outcome measure for disease progression.

Strengths and limitations of this study

- ✓ CT are capable of acquiring muscle images of all patients within a short time, and are adaptable to patients who cannot keep still in MRI scanners, such as mentally retarded patients or those with claustrophobia.

- ✓ We refer to the results as net muscle volume, because we calculated a 14cm section of the middle part of the thigh.
- ✓ There are problems with X-ray exposure to patients
- ✓ We cannot evaluate the net muscle volume less than 300 cm3.
- ✓ We cannot distinguish muscle from connective tissue accurately.

Introduction

Muscle volume in patients with muscle disease is an index of disease progression and the efficacy of therapy. Although muscle volume can be measured in healthy people using CT [1], MRI [2], DXA (dual energy X-ray absorptiometry) [3], and bio-impedance analysis [4], muscle volumetry in patients with muscular dystrophies has not been conducted.

Patients' respiratory dysfunction and spinal scoliosis made it difficult to establish muscle volumetry using underwater weighting [5] or bio-impedance analysis [4]. Muscular tissue where muscular fibers were mixed with fat also caused difficulty distinguishing between the two components using imaging methods.

An increase of signal intensity of T1 weighted images which suggested increased fat in the muscle of patients with Duchenne muscular dystrophy was reported [6]. And the qualified MR grades and clinical functional grades progression were well correlated [7], but the muscle volumetry, in which the muscle volume decrement were shown in the patients with muscular distrophies during several years, were not established.

Estimation of the concentration of fat using DIXON sequence on MRI has been reported, however, the concentration of muscle was not calculated using this method and muscle volume was not estimated [8-10]

CSA (cross sectional area analysis) [11, 12] using CT showed that the muscular cross sectional area of the middle section of the thigh corresponded well to muscle power and volume; however, accurate muscle volume could not be estimated due to the coexistence of muscle fibers and fat tissue in the same muscle bundles of patients with

muscular dystrophies.

Although the histogram of CT values in the thigh muscle showed peaks in muscle CT values in non-neuromuscular disease patients or very mild muscular dystrophy patients, the peak of the histogram shifted to fat CT values according to disease progression [13]. Intermediate CT values between fat and muscle CT values corresponded to the concentration ratio of muscle fibers and fat tissue in muscle tissue. Recently, the main object of muscle volumetry was to distinguish muscle from fat [12, 13], and voxels which showed intermediate CT values were not investigated.

The method to distinguish the two tissues by standard CT values may underestimate muscle volume, because muscle tissue which was mixed with fat tissue and represented lower CT values than standard muscle CT values was totally excluded [12] (Figure 1). The method to separate muscle from fat anatomically also did not deal with muscle tissue mixed with fat because the method was developed for healthy people or athletes [13].

In this study, we proposed a method of estimating muscle volume in muscle tissue mixed with fat tissue using CT in patients with muscular diseases. We determined standard CT values of muscle and fat in helical CT and developed a new method to measure muscle volume using our estimation function in patients' thigh muscles, where skeletal muscle mainly contributes to daily functions, such as walking, and could be imaged easily using CT. We compared results from this new method to DXA analysis and the disability classification of the muscle disease. Finally, we modified the method to a long-term applicable method by calculating constant zones. As we limited the calculating partition and the results were not accurate thigh muscle volumes, we referred to this method as *net* muscle volumetry. In order to ascertain the usefulness of this novel method of diseased muscle volumetry, we prospectively followed 14 muscular dystrophy patients and recognized progressive net muscle volume decreases.

Subjects

1. Standard CT values of muscle

Abdominal CT images of 114 non-neuromuscular disease patients, who had been scanned using CT for abdominal pain using a TOSHIBA multi-detector CT scanner, aged between 20 and 30 years old (average 24 +/- 3.52(SD)) were used to evaluate muscle and fat CT values in Yokohama Rosai Hospital. They were studied according to the Human Research Guidelines of the Internal Ethical Review Board of Yokohama Rosai Hospital. Abdominal organs and muscles surrounding the abdominal cavity were not obviously impaired in these images. The section that an umbilicus was included in was evaluated.

2. Muscle volumetry using CT and DXA

Thirteen patients (facioscapulohumeral muscular dystrophy (FSH): 4, type l myotonic dystrophy (MyD): 6, limb-girdle muscular dystrophy (LGMD): 2, inclusion body myositis (IBM): l), (male 11, female 2) aged between 19 and 67 years old (average: 52.7 +/- 14.7(SD)) participated in this study in 2007 at Yokohama Rosai Hospital, NHO Suzuka Hospital, NHO Shimoshizu Hospital, and NHO Higashisaitama Hospital. All patients were clinically or pathologically diagnosed. They were studied according to the Human Research Guidelines of the Internal Ethical Review Board of Yokohama Rosai Hospital and the National Hospital Organization. They agreed to this study and assigned with their signature. Their clinical disability stage was determined on the Ueda's motor disability classification, which was widely used in patients with muscular dystrophies in Japan [14]. The original report of UEDA disability classification was published in Japanese and was not listed on PubMed database, therefore we showed the correspondence list of the UEDA functional disability stage and the leg grading scale of Vignos functional rating scale (Table 1) [15]. A priori number of patients analyzed by correlation coefficient was estimated using G*power (http://www.psycho.uni-duesseldorf.de/abteilungen/aap/gpower3/; effect size = 0.5, α error probability = 0.05, power = 0.8), and it was presumed as 26

limbs of 13 patients.

3. Prospective net muscle volumetry using CT

Twelve patients (FSH: 3, MyD: 3, bulbospinal muscular atrophy: 2, IBM: 2, LGMD: 1, periodic paralysis 1), (male 9, female 3) aged between 31 and 66 years old (50.67 +/- 13.1(SD)) participated for 4 years (2007-2010) at Yokohama Rosai Hospital and NHO Suzuka Hospital. They also agreed to participate in our study.

Methods

1. Standard CT values of muscle

To determine standard CT values of muscle and fat, muscular CT values of paravertebral and iliopsoas muscles and fatty CT values of subcutaneous fat were obtained.

2a. Muscle volumetry using CT

CT Data Acquisition

Data were acquired by TOSHIBA® and HITACHI® multi detector CT scanners (from 4 to 64 detectors). CT imaging was performed between the great trochanter and patella with a 1-cm slice thickness, 512x512 matrix, and 120 kV tube voltage.

CT Analysis

1) DICOM files between the great trochanter and patella were obtained from the image server.

2) According to our standard CT values of muscle and fat, the minimum (min) standard CT value, which was the average minus 2 SD (standard deviation), and maximum (max) standard CT value, which was the average plus 2 SD, were determined for each tissue. Voxels with CT values between the min and max standard muscle CT value and between the min and max standard fat CT value were assumed to be muscle and fat tissue, respectively. The other voxels with CT values between the max standard fat CT value and min standard muscle CT

value, or between the max standard muscle CT value and max standard muscle CT value plus (the min standard muscle CT value minus max standard fat CT value) were assumed to be the complex tissue of muscle and fat, or muscle and other tissues, respectively. We estimated muscle density function as muscle ratio=

(CT value of the voxel - max standard fat CT value)/(min standard muscle CT value - max standard fat CT value)
[max standard fat CT value =<CT value of the voxel < min standard muscle CT],

1
[min standard muscle CT =<CT value of the voxel < max standard muscle CT], and

(- CT value of the voxel + max standard muscle CT+(min standard muscle CT value - max standard fat CT value))/(min standard muscle CT value - max standard fat CT value)
[max standard muscle CT =<CT value of the voxel < max standard muscle CT+(min standard muscle CT value - max standard fat CT value)]. (Figure 2)
On MATLAB®, we applied the estimated function to the DICOM image, assigned the obtained muscle density to an 8 bit scale, and wrote an 8 bit gray scale TIFF image file. (Figure 3)

3) Obviously different structures from muscle were eliminated on the density map using Adobe Photoshop®.

4) Post processing density map files were collected on MATLAB® and muscle volume was calculated from a total summation of muscle density multiplied by voxel size.

2b. Muscle volumetry using DXA

DXA Data Acquisition

Data were acquired by Lunar® pencil beam type DXA scanners (DPX-LIQ, GE Lunar) and whole body DXA scans was executed.

DXA Analysis

Muscle mass was calculated from manually segmented thigh parts of data on attached software with DXA (DPX-L software Version 1.3). We calculated fat, lean mass, and bone and regarded the lean mass as the muscle mass, according to a previous report [3].

3. Prospective net muscle volumetry using CT

To compare results over several years, muscle volumes of the 14cm section of the middle part of the thigh, between 7cm above and below the midpoint of the trochanter and patella, were measured for 4 years. We referred to this volume as net muscle volume.

We used SPSS® 20 for statistical analysis.

Results

1. Standard CT values of muscle

Standard CT values of muscle and fat were determined to be 56.3 +/- 11.3 (2 SD) and -98.3 +/- 22.8 (2 SD), respectively.

2. Muscle volumetry using CT and DXA

Muscle volume of the thigh on one side was calculated as between 300 and 3400 cm^3 by CT, and these volumes correlated to the muscle strength of these legs. Muscle mass of the thigh calculated from DXA was also estimated at between 1100 and 5000 g. A regression line between muscle mass and muscle volume estimated that: muscle mass (g) = 1.23 x muscle volume (cm^3) + 731 (g). The two results closely corresponded to one another with a Pearson's correlation coefficient of 0.993 (p=0.000), an interclass correlation coefficient (ICC(3, 1)) of 0.970, and a standard error of measurement (SEM) of 79.06 cm^3 in a Bland-Altman plot.

(Figure 4)

As the Ueda's disability classification of the lower extremities worsened, standardized muscle volume, which were muscle volume of thigh divided by square of body height[16], decreased with a Spearman's correlation coefficient of 0.767 (p=0.000). (Figure 5) In addition, the ratio of muscle volume divided by the distance between the trochanter and patella was highly correlated with the muscle square at the center of the thigh, with a Pearson's correlation coefficient of 0.978 (p=0.000). A regression line between muscle volume of the thigh and central muscle area estimated that: muscle volume of the thigh (cm^3) = (12.1 x central cross sectional muscle area – 9.66) x length between the great trochanter and patella

3. Prospective net muscle volumetry using CT

Net thigh muscle volume in 7 patients who complained of gait disturbance decreased in this period. The rate of decrease was 8.54 %/year (p=0.000 by a paired t-test). This rate led to a 41.0 % reduction in muscle volume in ten years. Net volume in 5 patients, who did not complain, did not decline (p=0.372 by a paired t-test, reduction rate was 0.39 %/year).

Discussion

A method to measure muscle volume based on CT, which was validated by muscle mass calculated using DXA, was developed. It also validated this method that the decrease of muscle volume was closely related to the aggravation of muscle functional classification. The inclination of the regression line between muscle volume from CT and muscle mass from DXA was calculated as 1.23 and represented the density of the muscle. It closely followed the specific gravity of the muscle, which was 1.17 [17], and also supported the probability of the calculation results. Demonstration of muscle volume decrease or consistency during four years also supported the reliability of this method.

The CT scanner is a widely used imaging tool and muscle images can be obtained in a short time. Although there are problems with X-ray exposure for patients, we can scan muscle CT images of a patient who cannot keep still in MRI scanners, such as mentally retarded patients or those with claustrophobia.

In this study, muscle was not anatomically or manually distinguished from other tissues, but was mostly automatically differentiated with the estimating function applying CT values of image pixels. In contrast, in DXA analysis, it is easy to make an error during manual procedures of segmentation of the thigh from the trunk. It was suggested that our approximately automatic process was superior to DXA analysis. This less arbitrary method is suitable for assessment of muscle volume in patients with muscular dystrophy under clinical treatment trials and will become a strong clinical outcome measure for disease progression of patients with muscular dystrophies. However, there was limitation of this net volumetry. We cannot evaluate the net muscle volume less than 300 cm3, because in the images of the patients whose net muscle volume under 300 cm3, muscle tissue was almost changed to fat.

In this prospective *net* muscle volumetry, we calculated a 14cm section of the middle part of the thigh, between 7cm above and below the midpoint of the trochanter and patella, thereby minimizing spatial errors. For muscle volume, when measuring all sections between the trochanter and patella, the elimination of genital organs and tendons may lead to manual errors between examiners. On repeated examination, a difference in the number of slices between scans may occur, and a difference in the number of slices may cause fluctuations in muscle volume by several percentage (1/30 - 1/20, i.e. 3-5 %). On the other hand, the summation of a few slices will lead to positional differences between scans; therefore, we confirmed the number of slices as much as possible from our experiences. In addition, we decided to calculate the sections, because the diameter of the thigh was not so different between upper and bottom slices at the middle part of the thigh.

Slice thickness was determined as 10 mm similarly to cadaver thigh MRI and CT

scans [18] and proportionally to 3 mm slice thickness in mice volumetry using CT [19]. To minimize manual procedures, the numbers of slices should be reduced and slice thickness should be as thick as possible.

We determined the estimating linear function in the form of linear symmetry and as a median with muscle CT values on the histogram of CT values of images, because muscle and fat peaks on the histogram of CT values of non-muscular disease patients seemed to be normally distributed [20].

Although decreases or consistencies in net muscle volume were measured by our method, the accurate separation of muscle from connective tissue was impossible for the similar CT values of muscle and connective tissue. It was supposed that muscle tissue experiences fatty changes, but intramuscular connective tissue may not significantly increase within a year.

In addition, for child patients, evaluation of disease progression will be estimated by the residual muscle ratio: net muscle volume divided by net muscle volume + intrafascial fat volume in the middle part of the thigh, where muscle cross sectional area (CSA) was closely correlated to muscle volume of the thigh [12].

Finally, as muscle volume was different between patients according to their height, weight, and other factors, muscle volumes must be used not for comparison between patients, but as an index of the efficacy of therapies or of the progression of disease in one patient.

Acknowledgements

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(2882 words)

Contributorship Statement

T Nakayama, S Kuru, Y Motoyoshi and M Kawai contributed to all of this study.
M Okura contributed to the muscle volumetry using DXA.

Data Sharing

There are not any persons who share our data or our additional unpublished data.

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Ueda's motor disability classification based on Japanese lifestyle		The leg grading scale of Vignos functional rating scale	
Stage 1	Able to walk: Able to climb the stairs without assistance (without using a handrail)	Grade 1	Walks and climbs stairs without assistance.
Stage 2	Assistance (e.g., handrail) is necessary for climbing the stairs.	Grade 2	Walks and climbs stairs with aid of railing.
		Grade 3.	Walks and climbs stairs slowly with aid of railing (over 25 seconds for eight standard steps).
Stage 3	Unable to climb stairs: able to walk on a flat surface: able to stand up from a normal-height chair	Grade 4	Walks unassisted but cannot climb stairs or get out of chair.
Stage 4	Able to walk on a flat surface: unable to stand up from a chair	Grade 5	Walks unassisted but cannot rise from chair or climb stairs.
Stage 5	Unable to walk: able to crawl on four limbs	Grade 6	Walks only with assistance or walks independently with long leg braces.
Stage 6	Unable to crawl on four limbs but able to crawl in another pattern	Grade 7	Walks in long leg braces but requires assistance for balance.
Stage 7	Unable to crawl but able to maintain a sitting position by oneself	Grade 8	Stands in long leg braces but unable to walk even with assistance.
Stage 8	Unable to maintain a sitting position by oneself; total assistance is necessary	Grade 9	Wheelchair or bed bound; can only perform limited activities involving lower arm and hand muscles.

Table 1: Ueda's motor disability classification based on Japanese lifestyle and the leg grading scale of Vignos functional rating scale

Figure Legends

Figure 1: Estimation of muscle volume using only standard CT values

The voxels, whose CT values were between 40 and 65, were estimated for muscle tissue according to a previously reported method. This patient with progressive muscular dystrophy was able to walk with a cane, but he could not walk obviously in this presumed muscle tissue image.

Figure 2: Estimation of muscle volume from voxels

This figure showed an estimation of muscle volume from voxels where muscle and fat coexists. CT values of voxels, composed of muscle and fat, depended on the ratio of the two components. The histogram showed CT values of the thigh, and the two peaks of fat and muscle were shown. At a CT value of -75, the pixel included 0% muscle, and at a CT value of 45, the pixel included 100% muscle. The two points were connected with a linear function, this was then fit to the histogram, and, as a result, muscle density was obtained. This linear function was linearly symmetrically transferred to the other side of the muscle peak of the CT value.

Figure 3: Process of calculating muscle volume

These images were calculated from DIOOM data. Muscle is shown. We deleted obvious vessels and skin tissues manually from the density map, resulting in a muscle volume map of the thigh. Accumulation of outcomes resulted in muscle volume.

Figure 4: Correlation between muscle volumes calculated from CT and muscle mass from DXA

Results from DXA and CT are plotted. These results corresponded well with a Pearson's correlation coefficient of 0.993, interclass correlation coefficient (ICC(3,1)) of 0.970. The regression line was; muscle volume (CT) = 0.80 x muscle mass (DXA) - 561 (cm^3), muscle mass (DXA) = 1.23 x muscle volume (CT) + 731 (g), Bland Altman plot between the results of the regression function substituted by muscle

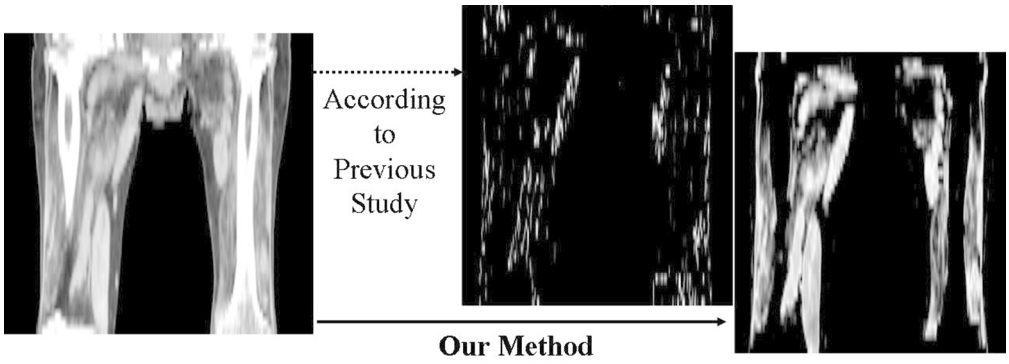
mass from DXA and the muscle volume calculated from CT showed no additional nor proportional error and a standard error of measurement(SEM) of 79.06 cm^3.

Figure 5: Correlation between muscle volume and disability stage

As Ueda's disability classification based on Japanese style in the lower extremities deteriorates, standardized muscle volume (muscle volume divided by square of body height) decreases with a Spearman's correlation coefficient of 0.767. The leg grading scale of Vignos functional rating scale was also shown.

Figure 6: Prospective net muscle volumetry in four years

During these four years, thigh muscle volumes in 7 patients who complained of gait disturbance decreased in this period (p=0.000). The rate of decrease was at a rate of 8.54 % / year. The volume in 5 patients, who did not complain, did not decline significantly (p=0.372).



Estimation of muscle volume using only standard CT values
The voxels, whose CT values were between 40 and 65, were estimated for muscle tissue according to a previously reported method. This patient with progressive muscular dystrophy was able to walk with a cane, but he could not walk obviously in this presumed muscle tissue image.
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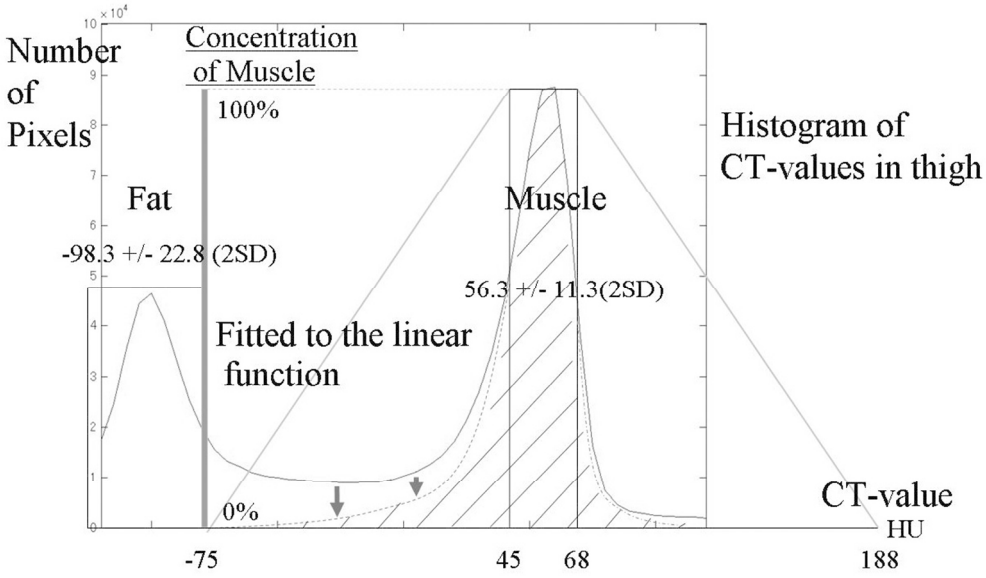

Estimation of muscle volume from voxels

This figure showed an estimation of muscle volume from voxels where muscle and fat coexists. CT values of voxels, composed of muscle and fat, depended on the ratio of the two components. The histogram showed CT values of the thigh, and the two peaks of fat and muscle were shown. At a CT value of -75, the pixel included 0% muscle, and at a CT value of 45, the pixel included 100% muscle. The two points were connected with a linear function, this was then fit to the histogram, and, as a result, muscle density was obtained. This linear function was linearly symmetrically transferred to the other side of the muscle peak of the CT value.

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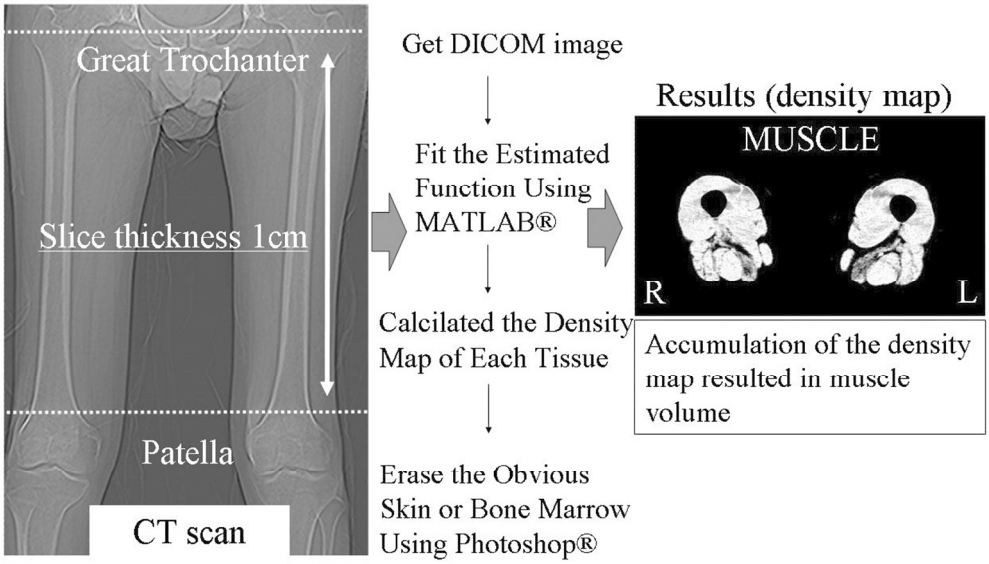
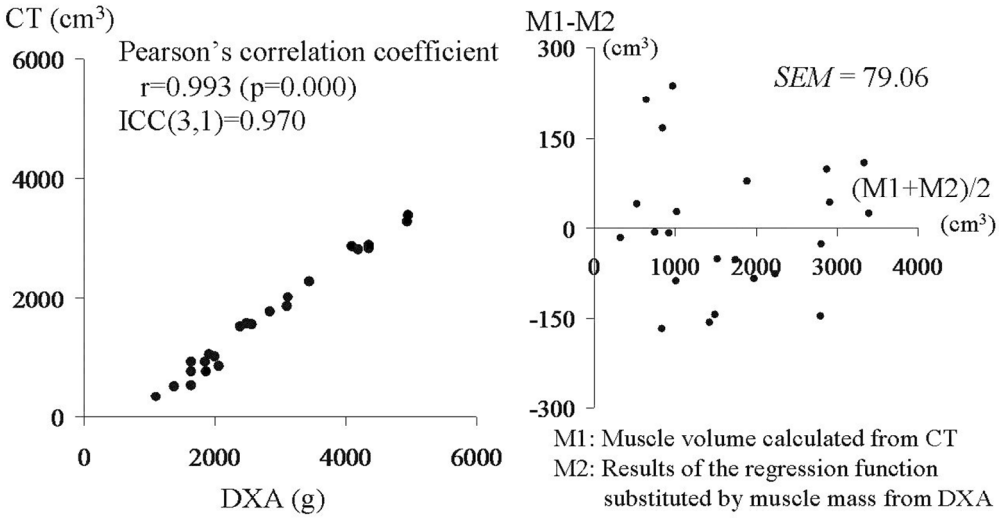


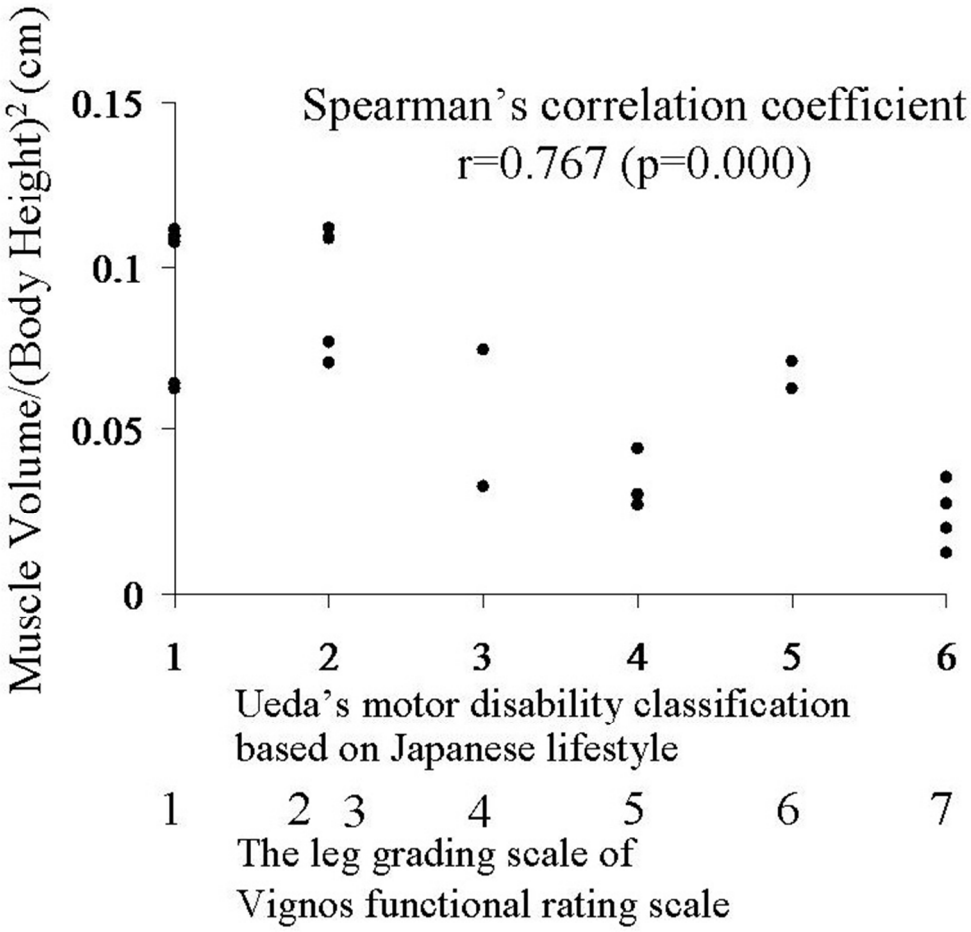
Process of calculating muscle volume
These images were calculated from DIOOM data. Muscle is shown. We deleted obvious vessels and skin tissues manually from the density map, resulting in a muscle volume map of the thigh. Accumulation of outcomes resulted in muscle volume.
332x188mm (96 x 96 DPI)



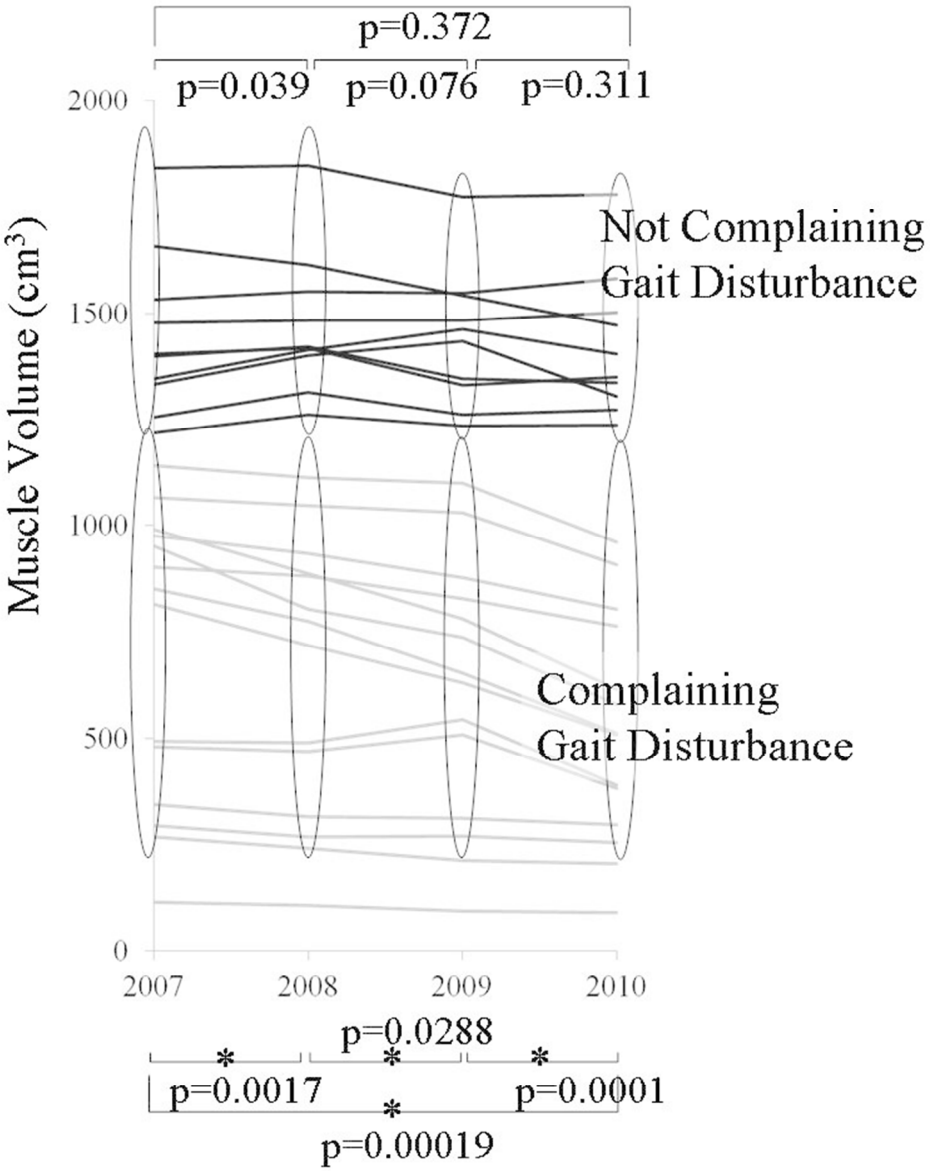
Muscle Volume (CT) = 0.80 x Muscle Mass (DXA) -561 (cm^3)

Muscle Mass (DXA) = 1.23x Muscle Volume (CT) +731 (g)

Correlation between muscle volumes calculated from CT and muscle mass from DXA
Results from DXA and CT are plotted. These results corresponded well with a Pearson's correlation coefficient of 0.993, interclass correlation coefficient (ICC(3,1)) of 0.970. The regression line was; muscle volume (CT) = 0.80 x muscle mass (DXA) - 561 (cm3), muscle mass (DXA) = 1.23 x muscle volume (CT) + 731 (g), Bland Altman plot between the results of the regression function substituted by muscle mass from DXA and the muscle volume calculated from CT showed no additional nor proportional error and a standard error of measurement(SEM) of 79.06 cm3.
338x201mm (96 x 96 DPI)



Correlation between muscle volume and disability stage
As Ueda's disability classification based on Japanese style in the lower extremities deteriorates, standardized muscle volume (muscle volume divided by square of body height) decreases with a Spearman's correlation coefficient of 0.767. The leg grading scale of Vignos functional rating scale was also shown.
205x194mm (96 x 96 DPI)



Prospective net muscle volumetry in four years
During these four years, thigh muscle volumes in 7 patients who complained of gait disturbance decreased in this period (p=0.000). The rate of decrease was at a rate of 8.54 % / year. The volume in 5 patients, who did not complain, did not decline significantly (p=0.372).
202x253mm (96 x 96 DPI)

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported
Objectives	3	State specific objectives, including any prespecified hypotheses
Methods		
Study design	4	Present key elements of study design early in the paper
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9	Describe any efforts to address potential sources of bias
Study size	10	Explain how the study size was arrived at
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy
		(*e*) Describe any sensitivity analyses

Continued on next page

Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time
		Case-control study—Report numbers in each exposure category, or summary measures of exposure
		Cross-sectional study—Report numbers of outcome events or summary measures
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results	18	Summarise key results with reference to study objectives
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	Discuss the generalisability (external validity) of the study results
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

Estimation of net muscle volume in patients with muscular dystrophy using muscle CT for prospective muscle volume analysis: observation study

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TITLE: Estimation of net muscle volume in patients with muscular dystrophy using muscle CT for prospective muscle volume analysis: observation study

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Key Words:
muscular dystrophy, muscle volumetry, outcome measure, CT, DXA

Word Count 3033 words
References: 22
Figures: 6, Table: 1

All correspondence should be sent to T. Nakayama addressed above.

Abstract

Objectives:

Muscle volume in patients with muscle disease is an index of disease progression. The aim of this study is to show a new method of muscle volumetry using CT of thigh muscles.

Design:

Observation study

Participants:

1. For muscle volumetry using CT and DXA, 13 patients with muscle disease participated.
2. For prospective CT volumetry, 12 patients participated for 4 years.

Primary and secondary outcome measures:

To establish the new CT volumetry, whose results were correlated to the muscle mass calculated from DXA (primary outcome). To show the decrease of muscle volume using our method over 4 years (secondary outcome)

Methods:

1. Helical CT imaging of the thigh was performed. CT images were analyzed applying estimated functions, and the accumulation of outcomes resulted in muscle volumes. We refer to this method as net muscle volumetry. Simultaneously, DXA was used in these patients and the muscle mass of the thigh was calculated by vendor provided software.
2. To evaluate longitudinal changes of muscle volumes, net muscle volumetry at 14-cm section of the middle part of thigh were performed repeatedly over a 4 years period.

Results:

1. Muscle volumes of the thigh on one side were calculated as between 300 and 3400 cm^3 by CT. Muscle masses of the thigh calculated from DXA were estimated at between 1100 and 5000 g. These results closely corresponded to each other with Pearson's correlation coefficient of 0.993.
2. Thigh net muscle volumes of 7 patients who complained of gait disturbance decreased over four years ($p<0.01$).

Conclusions:

Measurement of net muscle volume using CT, which was validated by the muscle mass calculated from DXA, was developed. Net muscle volume decrements over four years supported the reliability of this method. This less arbitrary method is suitable for assessment of muscle volume in patients with muscular dystrophy.

(300 words)

Article Summary

Article focus

- ✓ Muscle volume in patients with muscle disease is an index of disease progression and the efficacy of therapy, but muscle volumetry in which the muscle volume decreases could be shown in the patients with muscular dystrophies during several years has not been conducted thus far.
- ✓ There were some reports which showed estimated muscle volume were correlated to muscle power or functional rating scale in patients with muscular dystrophy using CT, MRI and DXA(dual energy X-ray absorptiometry).
- ✓ We proposed a method of muscle volumetry using our own estimating function to muscle CT images in patients with muscular diseases. And we demonstrated the long-term applicable method by calculating constant zones. And we called our method as net muscle volumetry.
- ✓ We measured muscle volume in patients' thigh muscles, where skeletal muscle mainly contributes to daily functions, using net muscle volumetry.

Key messages

- ✓ The muscle volume calculated by our method, that is net muscle volumetry, are closely correlated to the muscle mass using DXA, and the decrement of muscle volume are corresponded to the aggravation of muscle functional classification. These findings validated our method.
- ✓ We demonstrated the net muscle volume decrease or consistency over four years, and that also supported the reliability of this method.
- ✓ In this study, muscle was mostly automatically differentiated with the estimating function applying CT values of image pixels, except for manual elimination of skin or vessels. . This less arbitrary method is suitable for assessment of muscle volume in patients with muscular dystrophy under clinical treatment trials and will become a strong clinical outcome measure for disease progression.

Strengths and limitations of this study

- ✓ CT are capable of acquiring muscle images of all patients within a short time, and

are adaptable to patients who cannot keep still in MRI scanners, such as mentally retarded patients or those with claustrophobia.

- ✓ There are problems with X-ray exposure to patients
- ✓ We cannot evaluate the net muscle volume less than 300 cm3.
- ✓ We cannot distinguish muscle from connective tissue accurately.

Introduction

Muscle volume in patients with muscle disease is an index of disease progression and the efficacy of therapy. Although muscle volume can be measured in healthy people using CT [1], MRI [2], DXA (dual energy X-ray absorptiometry) [3], and bio-impedance analysis [4], muscle volumetry in patients with muscular dystrophies has not been conducted.

Patients' respiratory dysfunction and spinal scoliosis made it difficult to establish muscle volumetry using underwater weighting [5] or bio-impedance analysis [4]. Muscular tissue where muscular fibers were mixed with fat also caused difficulty distinguishing between the two components using imaging methods.

An increase of signal intensity of T1 weighted images which suggested increased fat in the muscle of patients with Duchenne muscular dystrophy was reported [6], and the qualified MR grades and clinical functional grades progression were well correlated [7]. Several groups have performed muscle volumetry in patients with muscular dystrophy on either single muscle or as whole body trials, however, none of these trials included longitudinal measurements of muscle volume [8, 9].

Estimation of the concentration of fat using DIXON sequence on MRI has been reported, however, the concentration of muscle was not calculated using this method and muscle volume was not estimated [10-12]

CSA (cross sectional area analysis) [13, 14] using CT showed that the muscular cross sectional area of the middle section of the thigh corresponded well to muscle power and volume; however, accurate muscle volume could not be estimated due to the coexistence of muscle fibers and fat tissue in the same muscle bundles of patients with

muscular dystrophies.

Although the histogram of CT values in the thigh muscle showed peaks in muscle CT values in non-neuromuscular disease patients or very mild muscular dystrophy patients, the peak of the histogram shifted to fat CT values according to disease progression [15]. Intermediate CT values between fat and muscle CT values corresponded to the concentration ratio of muscle fibers and fat tissue in muscle tissue. Recently, the main object of muscle volumetry was to distinguish muscle from fat [14, 15], and voxels which showed intermediate CT values were not investigated.

The method to distinguish the two tissues by standard CT values may underestimate muscle volume, because muscle tissue which was mixed with fat tissue and represented lower CT values than standard muscle CT values were totally excluded [14] (Figure 1). The method to separate muscle from fat anatomically also did not deal with muscle tissue mixed with fat because the method was developed for healthy people or athletes [15].

In this study, we proposed a method of estimating muscle volume in muscle tissue mixed with fat tissue using CT in patients with muscular diseases. We determined standard CT values of muscle and fat in helical CT and developed a new method to measure muscle volume using our estimation function in patients' thigh muscles, where skeletal muscle mainly contributes to daily functions, such as walking, and could be imaged easily using CT. We referred to this new method as *net* muscle volumetry. We compared results from this method to DXA analysis and the disability classification of the muscle disease. Finally, we modified the method to a long-term applicable method by calculating constant zones. In order to ascertain the usefulness of this novel method of diseased muscle volumetry, we prospectively followed 14 muscular dystrophy patients and recognized progressive net muscle volume decreases over 4 years.

Subjects

1. Standard CT values of muscle

Abdominal CT images of 114 non-neuromuscular disease patients, who had been scanned using CT for abdominal pain using a TOSHIBA multi-detector CT scanner; Aquilion 8 and 64, aged between 20 and 30 years old (average 24 +/- 3.52(SD)) were used to evaluate muscle and fat CT values at Yokohama Rosai Hospital. They were studied according to the Human Research Guidelines of the Internal Ethical Review Board of Yokohama Rosai Hospital. Abdominal organs and muscles surrounding the abdominal cavity were not obviously impaired in these images. We evaluated the CT image of constant level referred to an umbilicus of each patient.

2. Muscle volumetry using CT and DXA

All patients with muscular dystrophy presenting in 2007 were asked to participate our stydy, and thirteen patients (facioscapulohumeral muscular dystrophy (FSH): 4, type l myotonic dystrophy (MyD): 6, limb-girdle muscular dystrophy (LGMD): 2, inclusion body myositis (IBM): l), (male 11, female 2) aged between 19 and 67 years old (average: 52.7 +/- 14.7(SD)) participated in this study in 2007 at Yokohama Rosai Hospital, NHO Suzuka Hospital, NHO Shimoshizu Hospital, and NHO Higashisaitama Hospital. All patients were clinically or pathologically diagnosed. They were studied according to the Human Research Guidelines of the Internal Ethical Review Board of Yokohama Rosai Hospital and the National Hospital Organization. The patients gave written informed consent. Their clinical disability stage was determined on the Ueda's motor disability classification, which is widely used in patients with muscular dystrophies in Japan [16]. The original report of UEDA disability classification was published in Japanese and was not listed on PubMed database, therefore we showed the correspondence list of the UEDA functional disability stage and the leg grading scale of Vignos functional rating scale (Table 1) [17]. A priori number of patients analyzed by correlation coefficient was estimated using G*power (http://www.psycho.uni-duesseldorf.de/abteilungen/aap/gpower3/; effect size =

0.5, α error probability = 0.05, power = 0.8), and it was presumed as 26 limbs of 13 patients.

3. Prospective net muscle volumetry using CT

Twelve patients (FSH: 3, MyD: 3, bulbospinal muscular atrophy: 2, IBM: 2, LGMD: 1, periodic paralysis 1), (male 9, female 3) aged between 31 and 66 years old (50.67 +/- 13.1(SD)) participated for 4 years (2007-2010) at Yokohama Rosai Hospital and NHO Suzuka Hospital. They agreed to participate in our study.

Methods

1. Standard CT values of muscle

To determine standard CT values of muscle and fat, muscular CT values of paravertebral and iliopsoas muscles and fatty CT values of subcutaneous fat were obtained. ROIs were drawn at prespecified level including the whole muscle and mean ROI values were reported.

2a. Muscle volumetry using CT

CT Data Acquisition

Data were acquired by TOSHIBA® multi detector CT; Aquilion 8, 16 and 64 and HITACHI® multi detector CT; ROBUST (from 4 to 64 detectors). CT imaging was performed between the great trochanter and patella with a 1-cm slice thickness, 512x512 matrix, and 120 kV tube voltage.

CT Analysis

1) DICOM files between the great trochanter and patella were obtained from the image server. DICOM is a standard medical file format, and DICOM files contain patient data and images.

2) According to our standard CT values of muscle and fat, the minimum (min) standard CT value, which was the average minus 2 SD (standard deviation), and maximum (max) standard CT value, which was the average plus 2 SD, were determined for each tissue. Voxels with CT values between the min and max

standard muscle CT value and between the min and max standard fat CT value were assumed to be muscle and fat tissue, respectively. The other voxels with CT values between the max standard fat CT value and min standard muscle CT value, or between the max standard muscle CT value and max standard muscle CT value plus (the min standard muscle CT value minus max standard fat CT value) were assumed to be the complex tissue of muscle and fat, or muscle and other tissues, respectively. We estimated muscle density function as below.

$$\text{muscle ratio} = \frac{\textit{CT value of the voxel} - (F + 2 * F\_SD)}{A} \quad \left((F + 2 * F\_SD) =< \textit{CT value of the voxel} < (M - 2 * M\_SD)\right)$$

$$= 1 \quad \left((M - 2 * M\_SD) =< \textit{CT value of the voxel} < (M + 2 * M\_SD)\right)$$

$$= \frac{-\textit{CT value of the voxel} + (M + 2 * M\_SD) + A}{A} \quad \left((M + 2 * M\_SD) =< \textit{CT value of the voxel} < ((M + 2 * M\_SD) + A)\right)$$

F = mean fat CT value M = mean muscle CT value

F_SD = standard deviation of fat CT value M_SD = standard deviation of muscle CT value

A = (M - 2 * M_SD) - (F + 2 * F_SD)

M - 2 * M_SD = min standard muscle CT value

M + 2 * M_SD = max standard muscle CT value

F – 2 * F_SD = min standard fat CT value

F + 2 * F_SD = max standard fat CT value

(Figure 2)

On MATLAB®, we applied the estimated function to the DICOM image, assigned the obtained muscle density to an 8 bit scale, and wrote an 8 bit gray scale TIFF image file. (Figure 3) MATLAB® is a numerical computing

environment and programming language developed by MathWorks.

3) Obviously different structures from muscle were eliminated on the density map using Adobe Photoshop®.

4) Post processing density map files were collected on MATLAB® and muscle volume was calculated from a total summation of muscle density multiplied by voxel size. We referred to this muscle volume as net muscle volume.

2b. Muscle volumetry using DXA

DXA Data Acquisition

Data was acquired by Lunar® pencil beam type DXA scanners (DPX-LIQ, GE Lunar) and whole body DXA scans were executed.

DXA Analysis

Muscle mass was calculated from manually segmented thigh parts of data on attached software with DXA (DPX-L software Version 1.3). We calculated fat, lean mass, and bone and regarded the lean mass as the muscle mass, according to a previous report [3].

We statistically compared the two results by Peason's correlation coefficient and Bland –Altman plot. We referred to this muscle volume calculated by our new method as net muscle volume. We also compared the Ueda's disability classification of the lower extremities of the patients to their standardized net muscle volume, which were muscle volume of thigh divided by square of body height[18], and statistically tested by Spearman's correlation coefficient.

Simultaneously the correlation between the ratio of net muscle volume divided by the distance from the trochanter to patella and the muscle square at the center of the thigh, was tested with a Pearson's correlation coefficient and regression analysis.

3. Prospective net muscle volumetry using CT

To compare results over several years, muscle volumes of the 14cm section of the middle part of the thigh, between 7cm above and below the midpoint of the trochanter and patella, were measured over 4 years. We performed a scan once a year over a 4 year period.

We used SPSS® 20 for statistical analysis.

Results

1. Standard CT values of muscle

   Standard CT values of muscle and fat were determined to be 56.3 +/- 11.3 (2 SD) and -98.3 +/- 22.8 (2 SD), respectively.

2. Muscle volumetry using CT and DXA

   Muscle volume of the thigh on one side was calculated as between 300 and 3400 cm^3 by CT, and these volumes correlated to the muscle strength of these legs. Muscle mass of the thigh calculated from DXA was also estimated at between 1100 and 5000 g. A regression line between muscle mass and muscle volume estimated that: muscle mass (g) = 1.23 x muscle volume (cm^3) + 731 (g). The two results closely corresponded to one another with a Pearson's correlation coefficient of 0.993 (p=0.000), an interclass correlation coefficient (ICC(3, 1)) of 0.970, and a standard error of measurement (SEM) of 79.06 cm^3 in a Bland-Altman plot. (Figure 4)

   As the Ueda's disability classification of the lower extremities worsened, standardized muscle volume, which were muscle volume of thigh divided by square of body height[16], decreased with a Spearman's correlation coefficient of 0.767 (p=0.000). (Figure 5)

   In addition, the ratio of muscle volume divided by the distance between the trochanter and patella was highly correlated with the muscle square at the center of the thigh, with a Pearson's correlation coefficient of 0.978 (p=0.000). A regression line between muscle volume of the thigh and central muscle area estimated that: muscle volume of the thigh (cm^3) = (12.1 x central cross sectional muscle area – 9.66) x length between the great trochanter and patella

3. Prospective net muscle volumetry using CT

   Net thigh muscle volume in 7 patients who complained of gait disturbance

decreased in this period, but their functional classification was not changed. The rate of decrease was 8.54 %/year (p=0.000 by a paired t-test). At this rate, reduction of muscle volume of 41.0 % would be expected over a 10 year period. Net volume in 5 patients, who did not complain, did not decline (p=0.372 by a paired t-test, reduction rate was 0.39 %/year).

Discussion

A method to measure muscle volume based on CT, which was validated by muscle mass calculated using DXA, was developed. In addition, we could demonstrate that the decrease of muscle volume was closely related to the aggravation of muscle functional classification. The inclination of the regression line between muscle volume from CT and muscle mass from DXA was calculated as 1.23 and represented the density of the muscle. It closely followed the specific gravity of the muscle, which was 1.17 [19], and also supported the probability of the calculation results. Demonstration of muscle volume decrease or consistency during four years also supported the reliability of this method.

The CT scanner is a widely used imaging tool and muscle images can be obtained in a short time. Although there are problems with X-ray exposure for patients, we can scan muscle CT images of a patient who cannot keep still in MRI scanners, such as mentally retarded patients or those with claustrophobia. MRI would be preferable in children due to radiation dose. However, in adults with muscular dystrophy and a low life expectancy due to the natural course of the disease, CT might be a valuable alternative.

In this study, muscle was not anatomically or manually distinguished from other tissues, but was mostly automatically differentiated with the estimating function applying CT values of image pixels. In contrast, in DXA analysis, it is easy to make an error during manual procedures of segmentation of the thigh from the trunk. It was suggested that our approximately automatic process was superior to DXA analysis. Furthermore, this method can be automated in a simple way and might

therefore be superior to MRI evaluations, where extensive manual segmentation is necessary. This less arbitrary method is suitable for assessment of muscle volume in patients with muscular dystrophy under clinical treatment trials and will become a strong clinical outcome measure for disease progression of patients with muscular dystrophies. However, there was limitation of this net volumetry. We cannot evaluate the net muscle volume less than 300 cm3, because in the images of the patients whose net muscle volumes under 300 cm3, muscle tissue was almost changed to fat.

In this prospective *net* muscle volumetry, we calculated a 14cm section of the middle part of the thigh, between 7cm above and below the midpoint of the trochanter and patella, thereby minimizing spatial errors. For muscle volume, when measuring all sections between the trochanter and patella, the elimination of genital organs and tendons may lead to manual errors between examiners. On repeated examination, a difference in the number of slices between scans may occur, and a difference in the number of slices may cause fluctuations in muscle volume by several percentage (1/30 - 1/20, i.e. 3-5 %). On the other hand, the summation of a few slices will lead to positional differences between scans; therefore, we confirmed the number of slices as much as possible from our experiences. In addition, we decided to calculate the sections, because the diameter of the thigh was not so different between upper and bottom slices at the middle part of the thigh.

Slice thickness was determined as 10 mm similarly to cadaver thigh MRI and CT scans [20] and proportionally to 3 mm slice thickness in mice volumetry using CT [21]. To minimize manual procedures, the numbers of slices should be reduced and slice thickness should be as thick as possible.

We determined the estimating linear function in the form of linear symmetry and as a median with muscle CT values on the histogram of CT values of images, because muscle and fat peaks on the histogram of CT values of non-muscular disease patients seemed to be normally distributed [22].

Although decreases or consistencies in net muscle volume were measured by our

method, the accurate separation of muscle from connective tissue was impossible for the similar CT values of muscle and connective tissue. It was supposed that muscle tissue experiences fatty changes, but intramuscular connective tissue may not significantly increase within a year.

In addition, for child patients, evaluation of disease progression will be estimated by the residual muscle ratio: net muscle volume divided by net muscle volume + intrafascial fat volume in the middle part of the thigh, where muscle cross sectional area (CSA) was closely correlated to muscle volume of the thigh [14].

Finally, as muscle volume was different between patients according to their height, weight, and other factors, muscle volumes must be used not for comparison between patients, but as an index of the efficacy of therapies or of the progression of disease in one patient.

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(3033 words)

Data sharing

No additional data available

Competing Interests

T Nakayama, S Kuru, Y Motoyoshi and M Kawai didn't receive any research support fees from TANITA Body Weight Scientific Institute.

Funding

None

Contributorship

T Nakayama, S Kuru, Y Motoyoshi and M Kawai contributed to all of this study.

M Okura contributed to the muscle volumetry using DXA.

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Ueda's motor disability classification based on Japanese lifestyle		The leg grading scale of Vignos functional rating scale	
Stage 1	Able to walk: Able to climb the stairs without assistance (without using a handrail)	Grade 1	Walks and climbs stairs without assistance.
Stage 2	Assistance (e.g., handrail) is necessary for climbing the stairs.	Grade 2	Walks and climbs stairs with aid of railing.
		Grade 3.	Walks and climbs stairs slowly with aid of railing (over 25 seconds for eight standard steps).
Stage 3	Unable to climb stairs: able to walk on a flat surface: able to stand up from a normal-height chair	Grade 4	Walks unassisted but cannot climb stairs or get out of chair.
Stage 4	Able to walk on a flat surface: unable to stand up from a chair	Grade 5	Walks unassisted but cannot rise from chair or climb stairs.
Stage 5	Unable to walk: able to crawl on four limbs	Grade 6	Walks only with assistance or walks independently with long leg braces.
Stage 6	Unable to crawl on four limbs but able to crawl in another pattern	Grade 7	Walks in long leg braces but requires assistance for balance.
Stage 7	Unable to crawl but able to maintain a sitting position by oneself	Grade 8	Stands in long leg braces but unable to walk even with assistance.
Stage 8	Unable to maintain a sitting position by oneself; total assistance is necessary	Grade 9	Wheelchair or bed bound; can only perform limited activities involving lower arm and hand muscles.

Table 1: Ueda's motor disability classification based on Japanese lifestyle and the leg grading scale of Vignos functional rating scale

Figure Legends

Figure 1: Estimation of muscle volume using only standard CT values

The voxels, whose CT values were between 40 and 65, were estimated for muscle tissue according to a previously reported method [7]. This patient with progressive muscular dystrophy was able to walk with a cane, but he would be unable to according to this presumed muscle tissue image by the previous method.

Figure 2: Estimation of muscle volume from voxels

This figure showed an estimation of muscle volume from voxels where muscle and fat coexists. CT values of voxels, composed of muscle and fat, depended on the ratio of the two components. The histogram showed CT values of the thigh, and the two peaks of fat and muscle were shown. At a CT value of -75, the pixel included 0% muscle, and at a CT value of 45, the pixel included 100% muscle. The two points were connected with a linear function, this was then fit to the histogram, and, as a result, muscle density was obtained. This linear function was linearly symmetrically transferred to the other side of the muscle peak of the CT value.

Figure 3: Process of calculating muscle volume

These images were calculated from DIOOM data. Muscle is shown. We deleted obvious vessels and skin tissues manually from the density map, resulting in a muscle volume map of the thigh. Accumulation of outcomes resulted in muscle volume.

Figure 4: Correlation between muscle volumes calculated from CT and muscle mass from DXA

Results from DXA and CT are plotted. These results corresponded well with a Pearson's correlation coefficient of 0.993, interclass correlation coefficient (ICC(3,1)) of 0.970. The regression line was; muscle volume (CT) = 0.80 x muscle mass (DXA) - 561 (cm^3), muscle mass (DXA) = 1.23 x muscle volume (CT) + 731 (g), Bland Altman plot between the results of the regression function substituted by muscle

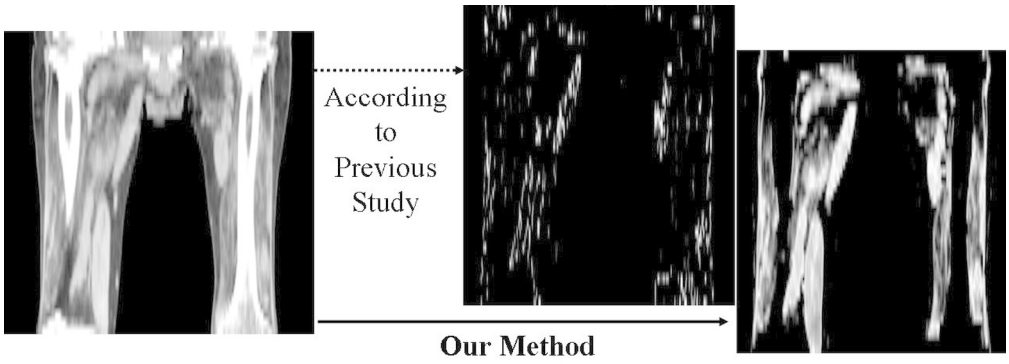
mass from DXA and the muscle volume calculated from CT showed no additional nor proportional error and a standard error of measurement(SEM) of 79.06 cm^3.

Figure 5: Correlation between muscle volume and disability stage

As Ueda's disability classification based on Japanese style in the lower extremities deteriorates, standardized muscle volume (muscle volume divided by square of body height) decreases with a Spearman's correlation coefficient of 0.767. The leg grading scale of Vignos functional rating scale was also shown.

Figure 6: Prospective net muscle volumetry in four years

During these four years, thigh muscle volumes in 7 patients who complained of gait disturbance decreased in this period (p=0.000). The rate of decrease was at a rate of 8.54 % / year. The volume in 5 patients, who did not complain, did not decline significantly (p=0.372). Samples of muscle density map of both patients who complain gait disturbance and who don't complain about gait disturbance were shown at the right side.



Estimation of muscle volume using only standard CT values

The voxels, whose CT values were between 40 and 65, were estimated for muscle tissue according to a previously reported method [7]. This patient with progressive muscular dystrophy was able to walk with a cane, but he would be unable to according to this presumed muscle tissue image by the previous method.
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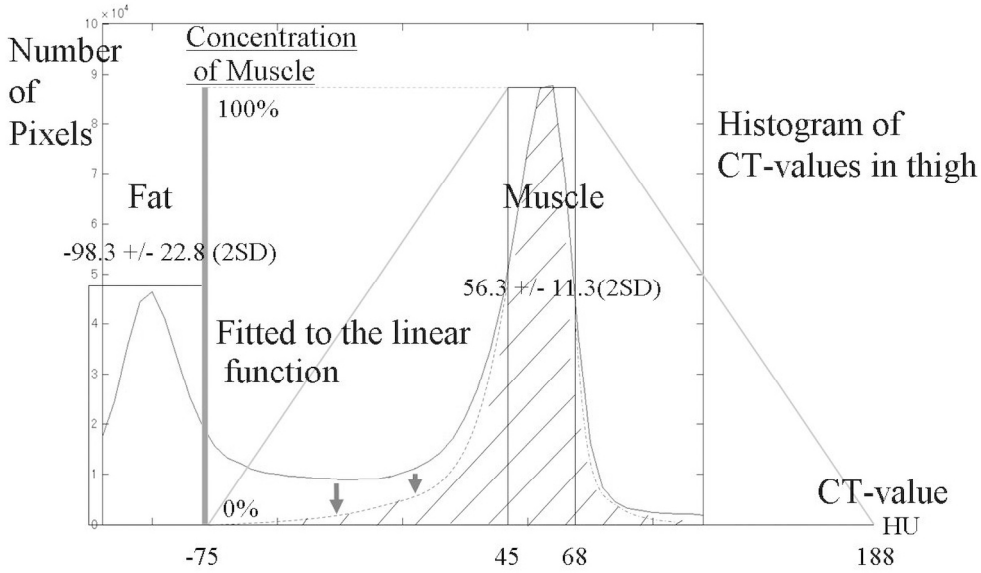

Estimation of muscle volume from voxels

This figure showed an estimation of muscle volume from voxels where muscle and fat coexists. CT values of voxels, composed of muscle and fat, depended on the ratio of the two components. The histogram showed CT values of the thigh, and the two peaks of fat and muscle were shown. At a CT value of -75, the pixel included 0% muscle, and at a CT value of 45, the pixel included 100% muscle. The two points were connected with a linear function, this was then fit to the histogram, and, as a result, muscle density was obtained. This linear function was linearly symmetrically transferred to the other side of the muscle peak of the CT value.

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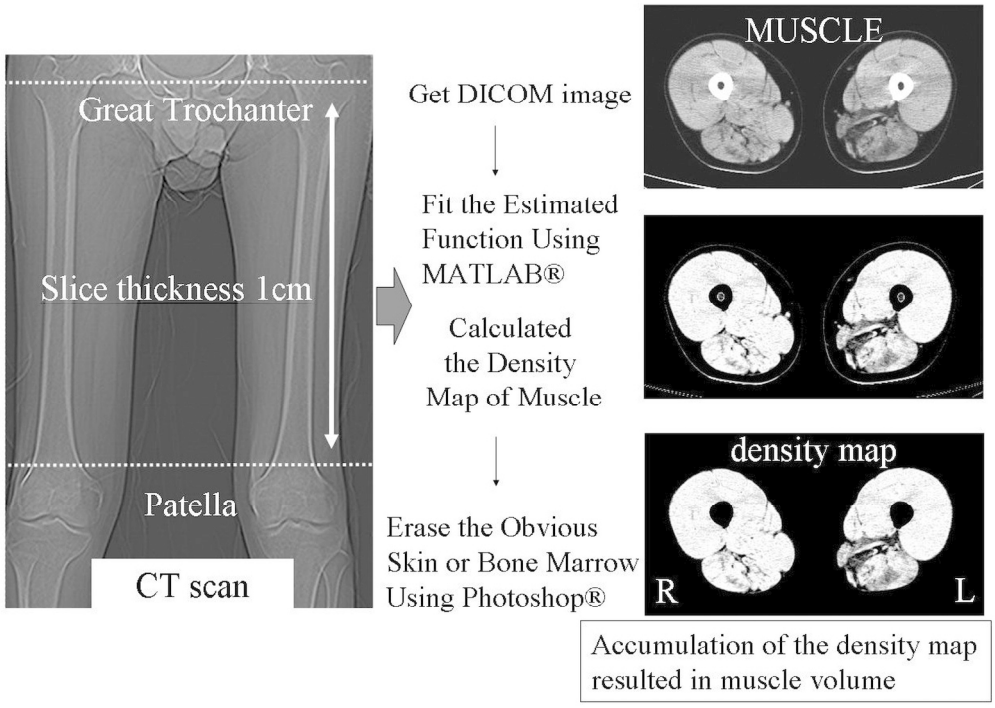

Process of calculating muscle volume

These images were calculated from DIOOM data. Muscle is shown. We deleted obvious vessels and skin tissues manually from the density map, resulting in a muscle volume map of the thigh. Accumulation of outcomes resulted in muscle volume.
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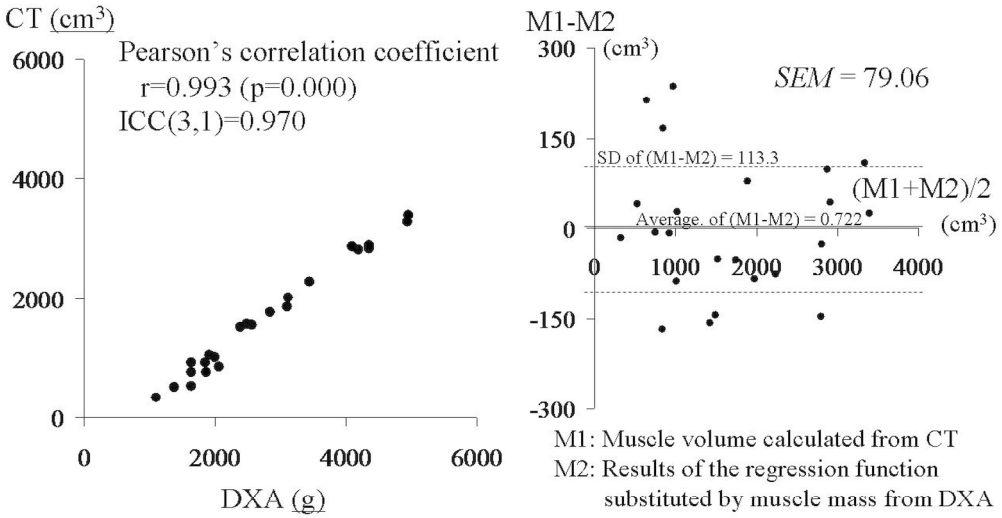

Correlation between muscle volumes calculated from CT and muscle mass from DXA

Results from DXA and CT are plotted. These results corresponded well with a Pearson's correlation coefficient of 0.993, interclass correlation coefficient (ICC(3,1)) of 0.970. The regression line was; muscle volume (CT) = 0.80 x muscle mass (DXA) - 561 (cm3), muscle mass (DXA) = 1.23 x muscle volume (CT) + 731 (g), Bland Altman plot between the results of the regression function substituted by muscle mass from DXA and the muscle volume calculated from CT showed no additional nor proportional error and a standard error of measurement(SEM) of 79.06 cm3.
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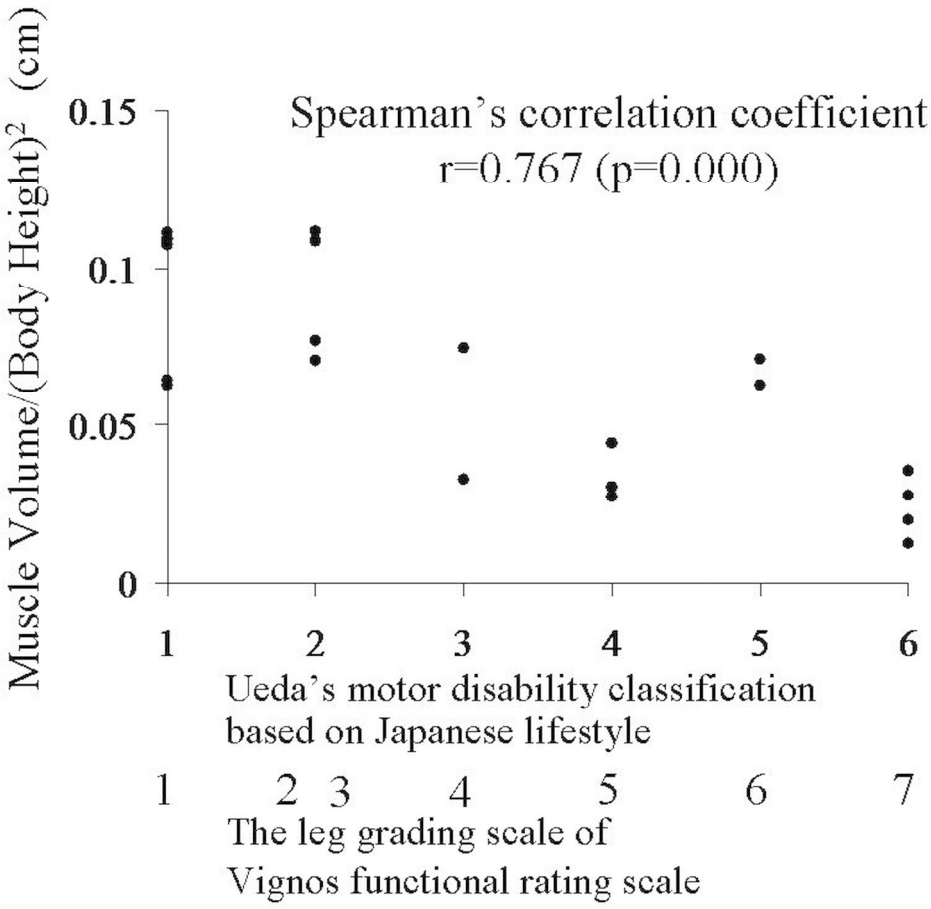

Correlation between muscle volume and disability stage

As Ueda's disability classification based on Japanese style in the lower extremities deteriorates, standardized muscle volume (muscle volume divided by square of body height) decreases with a Spearman's correlation coefficient of 0.767. The leg grading scale of Vignos functional rating scale was also shown.
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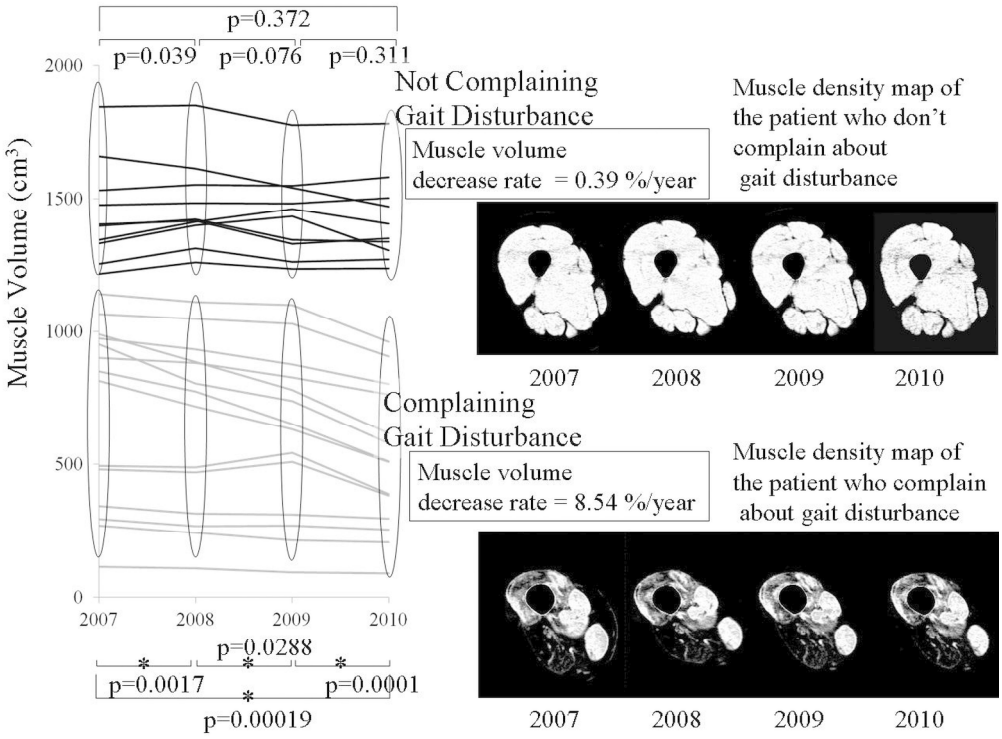

Prospective net muscle volumetry in four years

During these four years, thigh muscle volumes in 7 patients who complained of gait disturbance decreased in this period (p=0.000). The rate of decrease was at a rate of 8.54 % / year. The volume in 5 patients, who did not complain, did not decline significantly (p=0.372). Samples of muscle density map of both patients who complain gait disturbance and who don't complain about gait disturbance were shown at the right side.
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TITLE: ~~Measurement~~ Estimation of net muscle volume in patients with muscular dystrophy using muscle CT for prospective muscle volume analysis: observation study

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Key Words:
muscular dystrophy, muscle volumetry, outcome measure, CT, DXA

Word Count ~~2490~~ 3033 words
References: ~~16~~22
Figures: 6, Table: 1

All correspondence should be sent to T. Nakayama addressed above.

Abstract

Objectives:

~~:~~

Muscle volume in patients with muscle disease is an index of disease progression. The aim of this study is to show a new method of muscle volumetry using CT of thigh muscles.

Design:

Observation study

Participants:

~~Subjects~~

1. For muscle volumetry using CT and DXA, 13 patients with muscle disease participated.
2. For prospective CT volumetry, 12 patients participated for 4 years.

Primary and secondary outcome measures:

To establish the new CT volumetry, whose results were correlated to the muscle mass ~~measured by~~calculated from DXA (primary outcome). To show the decrease of muscle volume using our method ~~during~~over 4 years (secondary outcome)

Methods:

1.~~a.~~ ~~–~~Helical CT ~~scanner~~ imaging of the thigh was performed. CT images were analyzed applying estimated functions, and the accumulation of outcomes resulted in muscle volumes. We refer to this method as net muscle volumetry. Simultaneously, ~~,~~

~~1.b. Pencil beam~~ DXA was used in these patients and the muscle mass of the thigh was calculated by ~~the attached~~ vendor provided software.

2. ~~To prospectively compare muscle volumes, CT images of the mid-thigh were~~

measured. We referred to this method as net muscle volumetry. To evaluate longitudinal changes of muscle volumes, net muscle vvolumetry at 14--cm section of the middle part of thigh waswere performed repeatedly over a 4 years period.

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Results:

1. a. Muscle volumes of the thigh on one side were calculated as between 300 and 3400 cm^3 by CT.

1.b. Muscle masses of the thigh calculated from DXA were estimated at between 1100 and 5000 g. These Results results closely corresponded to each othermuscle volumes calculated by CT with Pearson'san interclass correlation coefficient of 0.993.

2. –Thigh net muscle volumes of 7 patients who complained of gait disturbance decreased in over four years ($p<0.01$).

Conclusions:

Measurement of net muscle volume using CT, which was validated by the muscle mass calculated from DXA, was developed. Net muscle volume decrements during over four years supported the reliability of this method. This less arbitrary method is suitable for assessment of muscle volume in patients with muscular dystrophy.

(250 300 words)

Article Summary

Article focus

- ✓ Muscle volume in patients with muscle disease is an index of disease progression and the efficacy of therapy, but muscle volumetry in which the muscle volume decreases could be shown in the patients with muscular dystrophies during several years has not been conducted thus~~so~~ far.
- ✓ There were some reports which showed estimated muscle volume were correlated to muscle power or functional rating scale in patients with muscular dystrophy using CT, MRI and DXA(dual energy X-ray absorptiometry).
- ✓ We proposed a method of muscle volumetry using our own estimating function to muscle CT images in patients with muscular diseases. And we demonstrated the long-term applicable method by calculating constant zones. And we called our method as net muscle volumetry.
- ✓ We measured muscle volume in patients' thigh muscles, where skeletal muscle mainly contributes to daily functions, using net muscle volumetry.

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Key messages

- ✓ The muscle volume ~~using~~ calculated by our method, that is net muscle volumetry, are closely correlated to the muscle mass using DXA, and the decrement of muscle volume are corresponded to the aggravation of muscle functional classification. These findings validated our method.
- ✓ We demonstrated the net muscle volume decrease or consistency ~~during~~ over four years, and that also supported the reliability of this method.
- ✓ In this study, muscle was mostly automatically differentiated with the estimating function applying CT values of image pixels, except for manual elimination of skin or vessels. . This less arbitrary method is suitable for assessment of muscle volume in patients with muscular dystrophy under clinical treatment trials and will become a strong clinical outcome measure for disease progression.

Strengths and limitations of this study

- ✓ CT are capable of acquiring muscle images of all patients within a short time, and

are adaptable to patients who cannot keep still in MRI scanners, such as mentally retarded patients or those with claustrophobia.

~~✓We refer to the results as net muscle volume, because we calculated a 14cm section of the middle part of the thigh.~~

✓ There are problems with X-ray exposure to patients

✓ We cannot evaluate the net muscle volume less than 300 cm3.

✓ We cannot distinguish muscle from connective tissue accurately.

Introduction

Muscle volume in patients with muscle disease is an index of disease progression and the efficacy of therapy. Although muscle volume can be measured in healthy people using CT [1], MRI [2], DXA (dual energy X-ray absorptiometry) [3], and bio-impedance analysis [4], muscle volumetry in patients with muscular dystrophies has not been conducted.

Patients' respiratory dysfunction and spinal scoliosis made it difficult to establish muscle volumetry using underwater weighting [5] or bio-impedance analysis [4]. Muscular tissue where muscular fibers were mixed with fat also caused difficulty distinguishing between the two components using imaging methods.

An increase of signal intensity of T1 weighted images which suggested increased fat in the muscle of patients with Duchenne muscular dystrophy was reported [6], and the qualified MR grades and clinical functional grades progression were well correlated [7]. Several groups have performed muscle volumetry in patients with muscular dystrophy on either single muscle or as whole body trials, however, none of these trials included longitudinal measurements of muscle volume [8, 9].

~~Hsieh et al. In vivo proton magnetic resonance spectroscopy assessment for muscle metabolism in neuromuscular diseases. J Pediatr (2007) vol. 151 (3) pp. 319-21~~

~~Gong et al. Estimation of body composition in muscular dystrophy by MRI and stereology. Journal of magnetic resonance imaging : JMRI (2000) vol. 12 (3) pp.~~

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~~467-75~~

~~but the muscle volumetry, in which the muscle volume decrement were shown in the patients with muscular distrophies during several years, were not established.~~

Estimation of the concentration of fat using DIXON sequence on MRI has been reported, however, the concentration of muscle was not calculated using this method and muscle volume was not estimated [~~8-10~~10-12]

CSA (cross sectional area analysis) [~~11~~13, ~~12~~14] using CT showed that the muscular cross sectional area of the middle section of the thigh corresponded well to muscle power and volume; however, accurate muscle volume could not be estimated due to the coexistence of muscle fibers and fat tissue in the same muscle bundles of patients with muscular dystrophies.

Although the histogram of CT values in the thigh muscle showed peaks in muscle CT values in non-neuromuscular disease patients or very mild muscular dystrophy patients, the peak of the histogram shifted to fat CT values according to disease progression [~~13~~15]. Intermediate CT values between fat and muscle CT values corresponded to the concentration ratio of muscle fibers and fat tissue in muscle tissue. Recently, the main object of muscle volumetry was to distinguish muscle from fat [~~12~~14, ~~13~~15], and voxels which showed intermediate CT values were not investigated.

The method to distinguish the two tissues by standard CT values may underestimate muscle volume, because muscle tissue which was mixed with fat tissue and represented lower CT values than standard muscle CT values wereas totally excluded [~~12~~14] (Figure 1). The method to separate muscle from fat anatomically also did not deal with muscle tissue mixed with fat because the method was developed for healthy people or athletes [~~13~~15].

In this study, we proposed a method of estimating muscle volume in muscle tissue mixed with fat tissue using CT in patients with muscular diseases. We determined standard CT values of muscle and fat in helical CT and developed a new method to measure muscle volume using our estimation function in patients' thigh muscles,

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where skeletal muscle mainly contributes to daily functions, such as walking, and could be imaged easily using CT. We referred to this new method as *net* muscle volumetry. We compared results from this ~~new~~ method to DXA analysis and the disability classification of the muscle disease. Finally, we modified the method to a long-term applicable method by calculating constant zones. ~~As we limited the calculating partition and the results were not accurate thigh muscle volumes, we referred to this method as *net* muscle volumetry.~~ In order to ascertain the usefulness of this novel method of diseased muscle volumetry, we prospectively followed 14 muscular dystrophy patients and recognized progressive net muscle volume decreases over 4 years.

Subjects

1. Standard CT values of muscle

~~2.~~

~~2.~~Abdominal CT images of 114 non-neuromuscular disease patients, who had been scanned using CT for abdominal pain using a TOSHIBA multi-detector CT scanner; Aquilion 8 and 64, aged between 20 and 30 years old (average 24 +/- 3.52(SD)) were used to evaluate muscle and fat CT values at~~in~~ Yokohama Rosai Hospital. They were studied according to the Human Research Guidelines of the Internal Ethical Review Board of Yokohama Rosai Hospital. Abdominal organs and muscles surrounding the abdominal cavity were not obviously impaired in these images. We evaluated the CT image of constant level ~~refferedreferred~~ to an umbilicus of each patient.

2. Muscle volumetry using CT and DXA

~~The section that an umbilicus was included in was evaluated.~~

~~2.Muscle volumetry using CT and DXA~~

All patients with muscular dystrophy presenting in 2007 were asked to participate our stydy, and ~~Thirteen~~ thirteen patients (facioscapulohumeral muscular dystrophy (FSH): 4, type l myotonic dystrophy (MyD): 6, limb-girdle muscular dystrophy

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(LGMD): 2, inclusion body myositis (IBM): l), (male 11, female 2) aged between 19 and 67 years old (average: 52.7 +/- 14.7(SD)) participated in this study in 2007 at Yokohama Rosai Hospital, NHO Suzuka Hospital, NHO Shimoshizu Hospital, and NHO Higashisaitama Hospital. All patients were clinically or pathologically diagnosed. They were studied according to the Human Research Guidelines of the Internal Ethical Review Board of Yokohama Rosai Hospital and the National Hospital Organization. The patients gave written informed consent. ~~They agreed to this study and assigned with their signature.~~ Their clinical disability stage was determined on the Ueda's motor disability classification, which ~~i~~was widely used in patients with muscular dystrophies in Japan [~~14~~16]. The original report of UEDA disability classification was published in Japanese and was not listed on PubMed database, therefore we showed the correspondence list of the UEDA functional disability stage and the leg grading scale of Vignos functional rating scale (Table 1) [~~15~~17]. A priori number of patients analyzed by correlation coefficient was estimated using G*power (http://www.psycho.uni-duesseldorf.de/abteilungen/aap/gpower3/; effect size = 0.5, α error probability = 0.05, power = 0.8), and it was presumed as 26 limbs of 13 patients.

~~4.~~3. Prospective net muscle volumetry using CT

Twelve patients (FSH: 3, MyD: 3, bulbospinal muscular atrophy: 2, IBM: 2, LGMD: 1, periodic paralysis 1), (male 9, female 3) aged between 31 and 66 years old (50.67 +/- 13.1(SD)) participated for 4 years (2007-2010) at Yokohama Rosai Hospital and NHO Suzuka Hospital. They ~~also~~ agreed to participate in our study.

Methods

1. Standard CT values of muscle

To determine standard CT values of muscle and fat, muscular CT values of

paravertebral and iliopsoas muscles and fatty CT values of subcutaneous fat were obtained. ROIs were drawn at prespecified level including the whole muscle and mean ROI values were reported.

2a. Muscle volumetry using CT

CT Data Acquisition

Data were acquired by TOSHIBA® multi detector CT; Aquilion 8, 16 and 64 and HITACHI® multi detector CT; ROBUST ~~scanners~~ (from 4 to 64 detectors). CT imaging was performed between the great trochanter and patella with a 1-cm slice thickness, 512x512 matrix, and 120 kV tube voltage.

CT Analysis

1) DICOM files between the great trochanter and patella were obtained from the image server. DICOM is a standard medical file format, and DICOM files contain patient data and images.

2) According to our standard CT values of muscle and fat, the minimum (min) standard CT value, which was the average minus 2 SD (standard deviation), and maximum (max) standard CT value, which was the average plus 2 SD, were determined for each tissue. Voxels with CT values between the min and max standard muscle CT value and between the min and max standard fat CT value were assumed to be muscle and fat tissue, respectively. The other voxels with CT values between the max standard fat CT value and min standard muscle CT value, or between the max standard muscle CT value and max standard muscle CT value plus (the min standard muscle CT value minus max standard fat CT value) were assumed to be the complex tissue of muscle and fat, or muscle and other tissues, respectively. We estimated muscle density function as below.

$$\text{muscle ratio} = \frac{CT\ value\ of\ the\ voxel - (F + 2 * F\_SD)}{A}$$

$$((F + 2 * F\_SD) =< CT\ value\ of\ the\ voxel < (M - 2 * M\_SD))$$

$$= 1$$

$$((M - 2 * M\_SD) =< CT\ value\ of\ the\ voxel < (M + 2 * M\_SD))$$

$$= \frac{-CT\ value\ of\ the\ voxel + (M + 2 * M\_SD) + A}{A}$$

$$((M + 2 * M\_SD) =< CT\ value\ of\ the\ voxel < ((M + 2 * M\_SD) + A))$$

F = mean fat CT value M = mean muscle CT value

F_SD = standard deviation of fat CT value M_SD = standard deviation of muscle CT value

A = (M - 2 * M_SD) - (F + 2 * F_SD)

M - 2 * M_SD = min standard muscle CT value

M + 2 * M_SD = max standard muscle CT value

F – 2 * F_SD = min standard fat CT value

F + 2 * F_SD = max standard fat CT value

$$muscle\ ratio = \frac{CT\ value\ of\ the\ voxel - \max\ standard\ \ fat\ CT\ value}{A}$$

$$muscle\ ratio = \frac{CT\ value\ of\ the\ voxel + \text{standard} \quad muscle\ CT\ value}{A} \quad A +$$

(Figure 2)

On MATLAB®, we applied the estimated function to the DICOM image, assigned the obtained muscle density to an 8 bit scale, and wrote an 8 bit gray scale TIFF image file. (Figure 3) MATLAB® is a numerical computing environment and programming language developed by MathWorks.

3) Obviously different structures from muscle were eliminated on the density map using Adobe Photoshop®.

4) Post processing density map files were collected on MATLAB® and muscle

volume was calculated from a total summation of muscle density multiplied by voxel size. We referred to this muscle volume as net muscle volume.

2b. Muscle volumetry using DXA

DXA Data Acquisition

Data w~~e~~as~~re~~ acquired by Lunar® pencil beam type DXA scanners (DPX-LIQ, GE Lunar) and whole body DXA scans were~~as~~ executed.

DXA Analysis

Muscle mass was calculated from manually segmented thigh parts of data on attached software with DXA (DPX-L software Version 1.3). We calculated fat, lean mass, and bone and regarded the lean mass as the muscle mass, according to a previous report [3].

We statistically compared the two results by Peason's correlation coefficient and Bland –Altman plot. We referred to this muscle volume calculated by our new method as net muscle volume. We also compared the Ueda's disability classification of the lower extremities of the patients to their standardized net muscle volume, which were muscle volume of thigh divided by square of body height[~~16~~18], and statistically tested by Spearman's correlation coefficient. Simultaneously the correlation between the ratio of net muscle volume divided by the distance fr~~o~~om the trochanter to patella and the muscle square at the center of the thigh, was tested with a Pearson's correlation coefficient and regression analysis.

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3. Prospective net muscle volumetry using CT

To compare results over several years, muscle volumes of the 14cm section of the middle part of the thigh, between 7cm above and below the midpoint of the trochanter and patella, were measured ~~for~~ over 4 years. We performed a scan once a year over a 4 year period. ~~We referred to this volume as net muscle volume.~~

We used SPSS® 20 for statistical analysis.

Results

1. Standard CT values of muscle

   Standard CT values of muscle and fat were determined to be 56.3 +/- 11.3 (2 SD) and -98.3 +/- 22.8 (2 SD), respectively.

2. Muscle volumetry using CT and DXA

   Muscle volume of the thigh on one side was calculated as between 300 and 3400 cm^3 by CT, and these volumes correlated to the muscle strength of these legs. Muscle mass of the thigh calculated from DXA was also estimated at between 1100 and 5000 g. A regression line between muscle mass and muscle volume estimated that: muscle mass (g) = 1.23 x muscle volume (cm^3) + 731 (g). The two results closely corresponded to one another with a Pearson's correlation coefficient of 0.993 (p=0.000), an interclass correlation coefficient (ICC(3, 1)) of 0.970, and a standard error of measurement (SEM) of 79.06 cm^3 in a Bland-Altman plot. (Figure 4)

   As the Ueda's disability classification of the lower extremities worsened, standardized muscle volume, which were muscle volume of thigh divided by square of body height[16], decreased with a Spearman's correlation coefficient of 0.767 (p=0.000). (Figure 5)

   In addition, the ratio of muscle volume divided by the distance between the trochanter and patella was highly correlated with the muscle square at the center of the thigh, with a Pearson's correlation coefficient of 0.978 (p=0.000). A regression line between muscle volume of the thigh and central muscle area estimated that: muscle volume of the thigh (cm^3) = (12.1 x central cross sectional muscle area – 9.66) x length between the great trochanter and patella

3. Prospective net muscle volumetry using CT

   Net thigh muscle volume in 7 patients who complained of gait disturbance decreased in this period, but their functional classification was not changed. The rate of decrease was 8.54 %/year (p=0.000 by a paired t-test). At ~~This~~ this

rate, reduction of muscle volume of ~~led to a~~ 41.0 % would be expected over a 10 year period~~reduction in muscle volume in ten years~~. Net volume in 5 patients, who did not complain, did not decline (p=0.372 by a paired t-test, reduction rate was 0.39 %/year).

Discussion

A method to measure muscle volume based on CT, which was validated by muscle mass calculated using DXA, was developed. In addition, we could demonstrate ~~t also validated this method~~ that the decrease of muscle volume was closely related to the aggravation of muscle functional classification. The inclination of the regression line between muscle volume from CT and muscle mass from DXA was calculated as 1.23 and represented the density of the muscle. It closely followed the specific gravity of the muscle, which was 1.17 [~~17~~19], and also supported the probability of the calculation results. Demonstration of muscle volume decrease or consistency during four years also supported the reliability of this method.

The CT scanner is a widely used imaging tool and muscle images can be obtained in a short time. Although there are problems with X-ray exposure for patients, we can scan muscle CT images of a patient who cannot keep still in MRI scanners, such as mentally retarded patients or those with claustrophobia. MRI would be preferable in children due to radiation dose. However, in adults with muscular dystrophy and a low life expectancy due to the natural course of the disease, CT might be a valuable alternative.

In this study, muscle was not anatomically or manually distinguished from other tissues, but was mostly automatically differentiated with the estimating function applying CT values of image pixels. In contrast, in DXA analysis, it is easy to make an error during manual procedures of segmentation of the thigh from the trunk. It was suggested that our approximately automatic process was superior to DXA analysis. Furthermore, this method can be automated in a simple way and might therefore be superior to MRI evaluations, where extensive manual segmentation is

necessary. This less arbitrary method is suitable for assessment of muscle volume in patients with muscular dystrophy under clinical treatment trials and will become a strong clinical outcome measure for disease progression of patients with muscular dystrophies. However, there was limitation of this net volumetry. We cannot evaluate the net muscle volume less than 300 cm3, because in the images of the patients whose net muscle volumes under 300 cm3, muscle tissue was almost changed to fat.

In this prospective *net* muscle volumetry, we calculated a 14cm section of the middle part of the thigh, between 7cm above and below the midpoint of the trochanter and patella, thereby minimizing spatial errors. For muscle volume, when measuring all sections between the trochanter and patella, the elimination of genital organs and tendons may lead to manual errors between examiners. On repeated examination, a difference in the number of slices between scans may occur, and a difference in the number of slices may cause fluctuations in muscle volume by several percentage (1/30 - 1/20, i.e. 3-5 %). On the other hand, the summation of a few slices will lead to positional differences between scans; therefore, we confirmed the number of slices as much as possible from our experiences. In addition, we decided to calculate the sections, because the diameter of the thigh was not so different between upper and bottom slices at the middle part of the thigh.

Slice thickness was determined as 10 mm similarly to cadaver thigh MRI and CT scans [~~18~~20] and proportionally to 3 mm slice thickness in mice volumetry using CT [~~19~~21]. To minimize manual procedures, the numbers of slices should be reduced and slice thickness should be as thick as possible.

We determined the estimating linear function in the form of linear symmetry and as a median with muscle CT values on the histogram of CT values of images, because muscle and fat peaks on the histogram of CT values of non-muscular disease patients seemed to be normally distributed [~~20~~22].

Although decreases or consistencies in net muscle volume were measured by our method, the accurate separation of muscle from connective tissue was impossible for

the similar CT values of muscle and connective tissue. It was supposed that muscle tissue experiences fatty changes, but intramuscular connective tissue may not significantly increase within a year.

In addition, for child patients, evaluation of disease progression will be estimated by the residual muscle ratio: net muscle volume divided by net muscle volume + intrafascial fat volume in the middle part of the thigh, where muscle cross sectional area (CSA) was closely correlated to muscle volume of the thigh [~~12~~14].

Finally, as muscle volume was different between patients according to their height, weight, and other factors, muscle volumes must be used not for comparison between patients, but as an index of the efficacy of therapies or of the progression of disease in one patient.

Acknowledgements

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(~~2882~~ 3033 words)

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Ueda's motor disability classification based on Japanese lifestyle		The leg grading scale of Vignos functional rating scale	
Stage	Able to walk: Able to climb	Grade 1	Walks and climbs stairs without assistance.
1	the stairs without assistance (without using a handrail)		
Stage 2	Assistance (e.g., handrail) is necessary for climbing the stairs.	Grade 2	Walks and climbs stairs with aid of railing.
		Grade 3.	Walks and climbs stairs slowly with aid of railing (over 25 seconds for eight standard steps).
Stage 3	Unable to climb stairs: able to walk on a flat surface: able to stand up from a normal-height chair	Grade 4	Walks unassisted but cannot climb stairs or get out of chair.
Stage 4	Able to walk on a flat surface: unable to stand up from a chair	Grade 5	Walks unassisted but cannot rise from chair or climb stairs.
Stage 5	Unable to walk: able to crawl on four limbs	Grade 6	Walks only with assistance or walks independently with long leg braces.
Stage 6	Unable to crawl on four limbs but able to crawl in another pattern	Grade 7	Walks in long leg braces but requires assistance for balance.
Stage 7	Unable to crawl but able to maintain a sitting position by oneself	Grade 8	Stands in long leg braces but unable to walk even with assistance.
Stage 8	Unable to maintain a sitting position by oneself; total assistance is necessary	Grade 9	Wheelchair or bed bound; can only perform limited activities involving lower arm and hand muscles.

Table 1: Ueda's motor disability classification based on Japanese lifestyle and the leg grading scale of Vignos functional rating scale

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Figure Legends

Figure 1: Estimation of muscle volume using only standard CT values

The voxels, whose CT values were between 40 and 65, were estimated for muscle tissue according to a previously reported method [7]. This patient with progressive muscular dystrophy was able to walk with a cane, but he would be unable to ~~could not walk obviously in~~ according to this presumed muscle tissue image by the previous method. ~~.~~

Figure 2: Estimation of muscle volume from voxels

This figure showed an estimation of muscle volume from voxels where muscle and fat coexists. CT values of voxels, composed of muscle and fat, depended on the ratio of the two components. The histogram showed CT values of the thigh, and the two peaks of fat and muscle were shown. At a CT value of -75, the pixel included 0% muscle, and at a CT value of 45, the pixel included 100% muscle. The two points were connected with a linear function, this was then fit to the histogram, and, as a result, muscle density was obtained. This linear function was linearly symmetrically transferred to the other side of the muscle peak of the CT value.

Figure 3: Process of calculating muscle volume

These images were calculated from DIOOM data. Muscle is shown. We deleted obvious vessels and skin tissues manually from the density map, resulting in a muscle volume map of the thigh. Accumulation of outcomes resulted in muscle volume.

Figure 4: Correlation between muscle volumes calculated from CT and muscle mass from DXA

Results from DXA and CT are plotted. These results corresponded well with a Pearson's correlation coefficient of 0.993, interclass correlation coefficient (ICC(3,1)) of 0.970. The regression line was; muscle volume (CT) = 0.80 x muscle mass (DXA) - 561 (cm^3), muscle mass (DXA) = 1.23 x muscle volume (CT) + 731 (g),

Bland Altman plot between the results of the regression function substituted by muscle mass from DXA and the muscle volume calculated from CT showed no additional nor proportional error and a standard error of measurement(SEM) of 79.06 cm^3.

Figure 5: Correlation between muscle volume and disability stage

As Ueda's disability classification based on Japanese style in the lower extremities deteriorates, standardized muscle volume (muscle volume divided by square of body height) decreases with a Spearman's correlation coefficient of 0.767. The leg grading scale of Vignos functional rating scale was also shown.

Figure 6: Prospective net muscle volumetry in four years

During these four years, thigh muscle volumes in 7 patients who complained of gait disturbance decreased in this period (p=0.000). The rate of decrease was at a rate of 8.54 % / year. The volume in 5 patients, who did not complain, did not decline significantly (p=0.372). Samples of muscle density map of both patients who complain gait disturbance and who don't complain about gait disturbance were shown at the right side.

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported
Objectives	3	State specific objectives, including any prespecified hypotheses
Methods		
Study design	4	Present key elements of study design early in the paper
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9	Describe any efforts to address potential sources of bias
Study size	10	Explain how the study size was arrived at
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy
		(*e*) Describe any sensitivity analyses

Continued on next page

Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time
		Case-control study—Report numbers in each exposure category, or summary measures of exposure
		Cross-sectional study—Report numbers of outcome events or summary measures
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results	18	Summarise key results with reference to study objectives
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	Discuss the generalisability (external validity) of the study results
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.