ある。そこで，本研究の第一の目的として，FFMの多い選手としてボディービルダーを対象にBMRの測定を行い，スポーツ選手における基礎代謝基準値について検討することとした。なお，BMRは，生命維持に必要な生理化学的反応を行うための覚醒安静時の最小エネルギーと定義され，実際には食後12時間以上経過した早朝空腹時に仰臥安静・覚醒状態で適正な室温において測定される[9,10]。しかし，食事摂取基準における基礎代謝基準値の算定根拠となったデータは，宿泊して起床後すぐに測定したデータと，測定当日に測定室に来室し30分程度の仰臥安静後，測定されたデータが混在している。そこで本研究においては，BMRを測定当日に測定場所へ来所した後移動し30分の仰臥安静後に空腹状態で測定した。また，引用する文献についても測定日の宿泊の有無を問わず，早朝空腹時に十分な安静後に測定した値をすべてBMRとして比較することとした。

PALについては，DRIでは二重標識水（Doubly Labeled Water：DLW）法を用いて測定したエネルギー消費量の測定値に基づいて，3段階に分類されている[1]。スポーツ選手のPALの設定にあたって，JISSのプロジェクトはスポーツ選手を対象とした先行研究におけるPALの値を基に，持久系，瞬発系，球技系，その他の種目カテゴリー別にオフトレーニング期と通常トレーニング期の期ごとにPALの値を示した[2,3]。瞬発系，球技系についてはスポーツ選手の値の平均値，持久系ではトレーニング時間が長いことと，体重が比較的軽い選手が多いことから既存研究のデータの上限値が採用されている。これまで測定されている選手のデータは，まだ限られた種目であり，特に日本人選手を対象としたものは少ない。そこで本研究の第二の目的として，レジスタンストレーニングを主として実施しているボディービルダーについてDLW法を使用してPALを測定し，スポーツ選手における身体活動レベルについて検討することとした。本研究で対象としたボディービルダーは，筋力の高度な発達を目的として，ほぼ毎日レジスタンストレーニングを実施している。トレーニング内容は他競技が筋力向上のために行っている内容とほぼ同じであるが，練習時間は他種目に比べ比較的短い対象である。

方　法

1．対象

対象者は，22～55歳の健康な成人男性で，週3～5回のトレーニングを行い，定期的に大会に参加しているボディービルダー14名である。全員，日本ボディービルディング連盟において薬物の使用がないことが確認されている。トレーニング量は週に平均4.6 ± 0.9回，1回のトレーニング時間は平均99 ± 21分で，週あたりの平均トレーニング時間は7.5 ± 2.4時間であった。測定期間は冬季であり，通常のトレーニング期であった。調査期間中は，できるだけ体重変動のない生活をするように指示し，それ以外は，通常の食事，トレーニングをするように指示した。

本研究は，独立行政法人国立健康・栄養研究所「人間を対象とする生物医学的研究に関する倫理委員会」の承認を得て，ヘルシンキ宣言の精神を遵守して実施した。被検者にはあらかじめ実験の目的と内容を説明し，文書により同意を得た。

2．測定項目

(1) 身体組成

身長及び体重は早朝空腹時に測定した。体脂肪率及びFFMはDXA法（Dual Energy X-ray Absorptiometry）（QDR－4500, Hologic, USA）により測定した。

(2) 基礎代謝量（BMR）

被験者は測定前夜の午後9時までに通常通りの夕食を済ませ，測定当日に朝食をとらずに測定場所に到着した後，9時から23～25℃の快適な室温で30分以上仰臥させた。測定場所への移動はできるだけ静かに行うように指示したが，移動による活動量は把握しなかった。その後，仰臥位のまま10分間の呼気を1分の間隔を置いてダグラスバッグに2回採集した。呼気は直ちに質量分析計（ARCO－1000，アルコシステム，千葉）を用いて酸素及び二酸化炭素量の濃度を分析した。その後，乾式ガスメーター（DC－5，シナガワ，東京）にて呼気量を測定した。それらの測定値から酸素摂取量（VO_2）と二酸化炭素排出量（VCO_2）を算出し，Weir[11]の式により1分あたりのBMRを求めた。さらに1,440（分）に換算し，1日あたりのBMR（kcal/day）とした。BMRは体重（Body Weight：BW）あたり（kcal/kg BW/day）及びFFMあたり（kcal/kg FFM /day）でも算出した。

(3) 身体活動レベル（PAL）の測定

対象者のうち7名について，DLW法を用いて総エネルギー消費量（Total Energy Expenditure：TEE）を測定し，それをBMRで除してPALを算出した。DLW法は，現時点では自由生活下での身体活動量を最も精度が高く評価できる方法とされており，ヒューマンカロリーメータとの比較により検討した正確度は約±4％とされている[12]。

10％ ^{18}O（太陽日酸，東京）と99.9％ ^{2}H（Cambridge Isotope Laboratories, Inc., USA）を混合した液により，体重あたり0.14gの ^{18}O と0.06gの ^{2}H を投与した。投与前，投与後4及び5時間後，翌日（2回）と8日後（2

Table 1 Physical characteristics and basal metabolic rate among body builders

	N	Age (yr.)	Height (cm)	BW (kg)	FFM (kg)	BMR (kcal)	BMR/BW (kcal/kg)	BMR/FFM (kcal/kg)
20－29yrs	3	24.0±3.5	172.3±7.6	80.0±11.5	70.7±11.0	1,843±264	23.0±0.4	26.1±1.2
30－39yrs	5	35.6±2.6	174.8±4.6	80.2±6.3	69.4±5.1	1,722±198	21.5±1.9	24.8±2.1
40－49yrs	5	41.8±2.5	169.5±6.4	72.6±6.4	65.0±6.0	1,590±171	21.9±1.4	24.5±1.7
50－59yrs	1	56	164.3	75.4	62.7	1,873	24.8	29.9
Total	14	36.8±9.1	171.6±6.2	77.1±7.6	67.6±6.8	1,712±209	22.2±1.6	25.4±2.1

BW：body weight, FFM：fat free mass assessed by dual energy X-ray absorptiometry, BMR：basal metabolic rate per day

回）の同時刻に採尿した。サンプルは密閉した状態で，分析まで－30℃で保存した。^{2}H は Pt を触媒として H_2 ガスで，^{18}O は CO_2 ガスで平衡法により前処理を行った後，^{2}H，^{18}O の安定同位体比を質量比分析計（Finnigan Delta Plus，Thermo Fisher Scientific，USA）により分析した。分析の測定誤差は，^{2}H で 0.5‰，^{18}O で 0.03‰である。また，10名のサンプルを2回分析した際の誤差は，1.6 ± 3.9％であった。

身体水分量（Total Body Water：TBW）は投与後4及び5時間後の尿中の安定同位体濃度から，N=［WA（δa－δt)］/［18.02a（δs－δb)］の式により求めた。ただし，N は ^{2}H 及び ^{18}O の希釈容積（mol)，W は同位体比分析の際に DLW を希釈するのに用いた飲料水の量（g)，A は投与した DLW の量（g)，δa は希釈した DLW における同位体比，δt は DLW の希釈に用いた飲料水の同位体比，a は同位体比分析の際に飲料水で希釈された DLW の量（g)，δs は尿中の同位体比，δb はベースラインでの尿の同位体比である。TBW は，^{2}H の N を 1.041 で除したものと，^{18}O の N を 1.007 で除したものの平均値とした。

測定期間中の安定同位体の減衰率を k=［ln（δf－δb）－ln（δi－δb)］/t から求めた。δf は8日後の尿中の同位体比，δb はベースライン尿の同位体比，δi は投与翌日の同位体比，t は測定期間である。二酸化炭素の排出量は，rCO_2（mol/day）=0.4554TBW（1.007ko－1.041kh）により求めた。ko は ^{18}O の減衰率，kh は ^{2}H の減衰率である。DLW 法においては，全期間を通じた呼吸商（Respiratory Quotient：RQ）の直接測定が不可能なため，体重変動のないエネルギーバランスのとれた状態では食事調査より求めた食物商（Food Quotient：FQ）[13] を使用して，TEE を求めることが最も適切とされている [14]。そこで，TEE は DLW 法による身体活動量の調査期間中の食事調査より求めた FQ を用いて，Weir[11] の式により求めた。

(4) 食事調査

PAL の測定を行った7名については，測定期間中に3日間の食事記録法により食事調査を行った。食事の記録内容は，調査終了後に管理栄養士が面接により確認した。摂取栄養素量は，エクセル栄養君 ver.4.0（建帛社，東京）により計算した。補助食品については，各メーカーの資料により栄養素量を求め，追加した。

(5) 統計処理

すべてのデータは，平均値と標準偏差（mean ± SD）で表した。本研究で得られた各指標の統計処理は，SPSS13.0 J for Windows (SPSS Inc., USA) にて行った。

結　　果

1. 基礎代謝量（BMR）

14名のボディービルダーの身体特性及び BMR を Table 1 に示した。全対象における1日あたりの BMR は，1日あたりでは 1,712 ± 209kcal/day，BW 1 kg あたりでは 22.2 ± 1.6kcal/kg/day，FFM あたりでは 25.4 ± 2.1kcal/kgFFM/day であった。年代別に分けると，各年代の人数は少ないものの，身体特性，BMR とも一定した傾向は認められなかった。

2. 身体活動レベル（PAL）

ボディービルダー7名の身体特性，BMR，TEE，PAL，歩数及び1週間あたりのトレーニング時間（分）を Table 2 に示した。DLW 法で測定した1日の TEE は，3,432 ± 634kcal/day であった。TEE と BMR から計算した PAL は，2.00 ± 0.21 であった。また，総エネルギー摂取量（Total Energy Intake：TEI）は1日あたりでは 3,268 ± 663kcal/day，体重あたりでは 43.3 ± 6.8kcal/kg/day であった。タンパク質，脂質，炭水化物の摂取量は，161 ± 55g，79 ± 25g，429 ± 130g，FQ は 0.923 であった。TEI と BMR から求めた TEI/BMR は 1.93 ± 0.24 であった。

考　　察

本研究で，高度にトレーニングされたボディービルダー男性の BMR を測定したところ，BMR/FFM は 25.4 ± 2.1kcal/kgFFM/day であり，JISS が設定した値（28.5kcal/kgFFM/day）[2,3] より低いことをみとめた。また，週に約8時間のトレーニングを行っているボデ

Table 2 Physical characteristics, basal metabolic rate, and physical activity level among body builders

ID	Age (yr.)	Height (cm)	BW-pre (kg)	BW-post (kg)	BW-change (kg)	FFM (kg)	BMR (kcal)	BMR/FFM (kcal/kg)	TEE (kcal)	PAL	Walk steps (steps/day)	Time (min)	TEI (kcal)
02	41	166.0	78.2	80.1	+1.8	69.9	1,845	26.4	4,191	2.20	10,870 ± 3,190	73	4,373
03	37	173.6	75.9	73.2	−1.7	63.8	1,741	27.3	3,884	2.23	13,903 ± 5,202	62	3,359
05	46	166.5	61.5	61.1	−0.3	55.2	1,385	25.1	2,421	1.81	12,759 ± 2,897	55	2,396
06	56	164.3	75.5	77.2	+1.7	62.7	1,873	29.9	3,228	1.78	5,374 ± 2,663	55	3,350
07	33	174.3	76.2	75.1	−1.1	65.5	1,555	23.7	2,965	1.81	–[a]	–[a]	2,533
09	42	163.4	73.7	72.6	−1.1	63.3	1,610	25.4	3,324	1.95	12,949 ± 3,016	46	3,280
11	39	181.6	90.0	89.1	−0.9	74.0	1,800	24.3	4,015	2.23	11,663 ± 2,655	34	3,587

BW：body weight, FFM：fat free mass assessed by dual energy X-ray absorptiometry, BMR：basal metabolic rate per day, TEE：total energy expenditure measured by doubly labeled water method, PAL：physical activity level calculated as TEE divided by BMR, Walk steps：mean value during TEE measurement assessed by accelerometer, Time：mean training time (minutes) per day during TEE measurement, TEI：total energy intake estimated by 3-day food records

[a] Values could not be assessed.

ィービルダーのPALは約2.0であった。

今回の結果では，FFM67.6kgのボディービルダーのBMR/FFMは25.4kcal/kgFFM/dayとなった。この値は，JISSが示した28.5kcal/kgFFM/day[2,3]や，先行研究におけるウォーキングまたはローイングをしているスポーツ愛好者男性（FFM52kg）28.5kcal/kgFFM/day[15]，水泳選手（FFM69kg）29.5kcal/kgFFM/day[16]，柔道選手（FFM67.1kg）28.1kcal/kgFFM/day[16]，空手選手（FFM64.5kg）28.2kcal/kgFFM/day[16]よりも，小さい値であった。FFMが35～45kgの者を対象とした研究[4,15,17]のBMR/FFMは，30～31kcal/kgFFM/dayと高い値が報告されていた。しかしながら，これらの先行研究では，FFMの測定法がBOD POD（空気置換法）[4,15]，DXA法[16,17]，水中体重法[17]と異なっている。また，BMRの測定条件や方法も，前日より宿泊してダグラスバッグにより測定したもの[4,15]，当日来所しフードを使用したもの[16]，当日あるいは前日に移動しフードを使用したもの[17]と様々であり，単純な比較は困難である。本研究と同じFFM，BMRの測定法による先行研究はなく，測定法による一定の傾向もみられなかった。

Weinsier, R. L. ら[9]のレビューによると，FFMが大きい対象においてBMR/FFMが小さくなることが指摘されている。その理由は，FFMが大きくなるとFFM中で安静時の代謝活性の低い筋組織の割合が安静時の代謝活性の高い内臓組織よりも大きくなるからであると指摘されている[5~8]。ボディービルディングでは，筋肉を高度にトレーニングしている。そのため，FFM中の筋肉の割合が非運動者や他の種目に比べて大きいことが推測され，FFMあたりの代謝率に影響する可能性は高い。一方で，Bosselares, I. ら[18]はヒューマンカロリーメータを使用して，ボディービルダーと非運動者の睡眠時代謝を比較し，年齢，FFM，体脂肪量（FM）で調整した睡眠時代謝には差がないとしている。また，Midorikawa ら[19]は水中体重法によりFFMを，MRIにより内臓の重量を測定し，肝臓と腎臓のFFMに占める割合は，運動群と非運動群でほぼ同じであり，FFMが大きくなってもその比率は減少しないという結果を得ている。脳・肝臓・心臓・腎臓の4器官は重量は体重の約6％しか占めないが，安静時の代謝量は約58％を占めており，そのうちでも肝臓と腎臓の代謝量は特に大きい[8]。しかし，FFMの量がBMRの違いに大きく影響していることは否定できず，選手の基礎代謝基準値としてBMR/BWよりもBMR/FFMを使用することは適切と考えられる。一方で，すべての選手に同一の値を使用できるかについては，今後，各種スポーツ選手について一定の方法でBMRとFFMを測定し，種目や体格などを考慮した基礎代謝基準値の設定が必要となるであろう。

TEEについては，齊藤らのレビュー[12]によると，スポーツ選手のPALは大学生女子水泳選手の1.71からツールドフランスのレース中の者の4.95となっている。日常的なトレーニングを行っていたスポーツ選手に限定すると，PALが2.2以内に75％の選手が分布する[20]。これまでに測定されたスポーツ選手のTEEに関する調査結果の平均値は2.03となったと報告されている[20]。本研究では7名のボディービルダーに対しDLW法による測定を行い，PALを算出したところ，2.00 ± 0.21となり，これらの報告とほぼ一致した。しかし，個人差は大きく1.78～2.23とばらついていた。この個人差の要因の1つは，個別のトレーニング内容には大きな差がないことから，1日あたりトレーニング時間が34～73分と倍以上の違いがあることによると推測される。しかしながら，Phillips, W. T. ら[21]の報告よりレジ

スタンストレーニング中の身体活動強度を3.9METs，運動時以外の平均を1.5METsとすると，30分のトレーニング時間の違いによるPALの差は（3.9－1.5）×30/1,440＝0.05となり，個人差を説明できるものではない。DLW法は自由生活下でのTEEを最も正確に評価できる方法とされているが，1～2週間の測定期間の1日の平均のTEEでしか評価できないという欠点がある。PALが2以上であった3名は，トレーニング指導員（2名）と技能職で仕事中も立位と歩行が多い作業であり，歩数も多い。一方で，最もPALの低い1名は歩数が少なく，仕事はほとんど座業でありトレーニング以外の身体活動量が極めて低かったことが推測される。ボディービルダーは特にトレーニング時間が短いが，それ以外のスポーツ選手でも合宿中などを除くと，トレーニング時間は限られている。トレーニング時間以外の生活における身体活動量による個人差を考慮しながら，どのように各種目のスポーツ選手の1日のPALを評価するか，今後，評価方法や基準となるPALの設定方法なども検討が必要であると考える。

以上より，スポーツ選手のBMRを測定した既存の資料では，FFM，BMRとも測定条件，方法などが異なり比較は困難であるが，今後，スポーツ選手の基礎代謝基準値の設定においては，種目や体格を考慮して示す必要があると考えられた。また，レジスタンストレーニングを主とするボディービルダーのPALは2.00±0.21であったが，個人差が大きく，スポーツ選手のPALの評価においては，トレーニング時間や内容の評価とそれ以外の時間の身体活動をどのように組み合わせて設定していくかが課題であると考えられた。

ま と め

22～55歳の高度にトレーニングを積んでいるボディービルダー14名を対象にBMR，FFM，PALを測定し，スポーツ選手のBMRとPALの基準値について検討した。

1）ボディービルダーの1日あたりのBMRは1,712±209kcal/dayであった。BMR/FFMは25.4±2.1kcal/kgFFM/dayであり，先行研究に比べると小さい傾向にあった。スポーツ選手の基礎代謝基準値の設定においては，その測定方法・条件を統一するとともに，種目や体格をどのように考慮するかを検討する必要があると考えられた。

2）DLW法で求めたボディービルダーの1日のTEEは3,432±634kcalで，PALは2.00±0.21であった。PALには個人差が大きく，PALの設定においては，トレーニング内容や時間の考慮だけでなく，それ以外の時間の身体活動量をどのように評価するか検討する必要があると考えられた。

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ORIGINAL ARTICLE

Validation of self-reported energy intake by a self-administered diet history questionnaire using the doubly labeled water method in 140 Japanese adults

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Objective: To validate reported energy intake (rEI) with a self-administered diet history questionnaire (DHQ) against total energy expenditure (TEE) by the doubly labeled water (DLW) method.
Subjects: A total of 140 healthy Japanese adults (67 men and 73 women) aged 20–59 years living in four areas in Japan.
Methods: Energy intake was assessed twice with DHQ over a 1-month period before and after TEE measurement (rEI_{DHQ1} and rEI_{DHQ2}, respectively). TEE was measured by DLW during 2 weeks (TEE_{DLW}).
Results: Mean rEI_{DHQ1} was lower than those of TEE_{DLW} by 1.9 ± 2.4 MJ/day (16.4%, $P<0.001$) for men and 0.6 ± 1.9 MJ/day (6.0%, $P<0.01$) for women. In men and women together, 62 subjects (44%) were defined as underreporters (rEI_{DHQ1}/TEE_{DLW} <0.84), 58 (41%) as acceptable reporters (0.84–1.16) and 20 (14%) as over-reporters (>1.16). Pearson correlation coefficient was 0.34 for men and 0.22 for women. After adjustment for the dietary and non-dietary factors related to rEI_{DHQ1}/TEE_{DLW}, the correlation coefficient improved to 0.42 and 0.37, respectively.
Conclusion: The energy intake assessed with DHQ correlated low to modestly with TEE measured by DLW. In addition, DHQ underestimated energy intake at a group level. Caution is needed when energy intake was evaluated by DHQ at both individual and group levels.
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Keywords: doubly labeled water; energy intake; self-administered diet history questionnaire; validation; Japanese adults

Introduction

Dietary intake estimates from self-administered dietary assessment methods such as questionnaires are commonly used in large-scale nutritional epidemiologic studies. Dietary assessment questionnaires have been developed for assessing habitual dietary intake and for ranking subjects according to their dietary intake. However, they cannot entirely avoid reporting errors (Barrett-Connor, 1991), including not only random but also systematic errors (Black and Cole, 2001; Livingstone and Black, 2003), due to the fact that they are self-reported.

In validation studies, data from dietary assessment questionnaires have often been compared with data from reference methods such as weighed diet records or 24 h recall (Willett and Lenart, 1998). However, all these dietary assessment methods were based on self-reporting. Therefore, the errors of both the new and reference methods might be correlated each other. The doubly labeled water (DLW) method, which measures the total energy expenditure (TEE) of subjects in free-living situations, has made it possible to validate reported energy intake (rEI) with an external biomarker (Hill and Davies, 2001; Trabulsi and Schoeller, 2001). The error of the DLW method is independent of self-rEI error (Livingstone and Black, 2003). However,

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relatively few validation studies of food frequency questionnaires against the DLW method have appeared (Sawaya *et al.*, 1996; Andersen *et al.*, 2003; Subar *et al.*, 2003). Furthermore, no such studies have been reported in non-Western countries.

The purpose of the present study was to examine the validity of energy intake assessed with a self-administered diet history questionnaire (DHQ) (Sasaki *et al.*, 1998) in comparison with TEE, as measured by the DLW method in a Japanese population.

Subjects and methods

Study population
This study was conducted in four districts of Japan from May to August 2003. We invited 40 healthy subjects (20 men and 20 women) aged 20–59 years from each of the four areas to participate, and distributed five subjects equally in each sex and age class of 20–29, 30–39, 40–49 and 50–59 years. Details of study recruitment and enrollment were described previously (Ishikawa-Takata *et al.*, 2007). All subjects providing written informed consent were finally considered eligible for the study. The total number of participants was 157 (78 men and 79 women).

Procedures
The study protocol was approved by the Ethics Committee of the National Institute of Health and Nutrition in Japan. The participants completed three visits over the study period and all participants completed the study. After recruitment, the participants were mailed an introductory letter and two dietary questionnaires including a DHQ, four physical activity questionnaires, and a supplemental questionnaire on lifestyle variables, and asked to fill them out and mail them back before the first visit (visit 1).

At visit 1, the participants had their questionnaires reviewed, their body weight and height measured and provided a baseline urine sample. At visit 2, on the morning following visit 1, they received a dose of DLW after an overnight fast. At visit 3, 14 days after visit 2, the participants brought urine samples and had their body weight and height measured.

After visit 3, the participants were mailed two dietary questionnaires including the DHQ, four physical activity questionnaires, supplemental questionnaire on lifestyle variables and diary about lifestyle during the period of TEE measurement.

All the collected questionnaires were checked by trained dietitians in each local center and again then in the study center. When missing answers, errors or both were found, the subjects were requested to answer the questions again.

Dietary assessment methods
Self-administered DHQ. The DHQ is a validated 16-page structured questionnaire, which assesses dietary habits in the preceding 1-month period (Sasaki *et al.*, 1998, 2000). Details of the questionnaire, methods of calculating nutrients and validity are given elsewhere (Sasaki *et al.*, 1998, 2000). Briefly, the DHQ consists of seven sections; (1) general dietary behavior, (2) major cooking methods, (3) consumption frequency and amount of six alcoholic beverages, (4) consumption frequency and semiquantitative portion size of 121 selected food and nonalcoholic beverage items, (5) dietary supplements, (6) consumption frequency and amount of 19 staple foods (rice, bread, noodles and other wheat foods) and miso soup (fermented soybean paste soup), and (7) open-ended items for foods consumed regularly (=once/week), which are not listed in the question. The food and beverage items and portion sizes in the DHQ were derived primarily from the data in the National Nutrition Survey of Japan (Sasaki *et al.*, 1998) and several recipe books for Japanese dishes. Measures of energy and dietary intakes for food and beverage items and dietary supplements with energy (148 food items in total) were calculated using an ad hoc computer algorithm for the DHQ, which was based on the Standard Tables of Food Composition in Japan (Science and Technology Agency, 2000). Information on dietary supplements, such as tablet, powder and liquid, which contained few energy and on data from the open-ended questionnaire items were not used in the calculation of dietary intake.

Anthropometric measures
Anthropometric measures were obtained at visits 1 and 3 by a single-trained study member. Body weight and height were measured to the nearest 0.1 kg and 0.1 cm, respectively, in subjects wearing light clothing and no shoes. body mass index (BMI) was calculated as body weight (kg) divided by the square of body height (m^2).

Measurement of TEE with the DLW method
At visit 2, after a baseline urine sample was obtained, a single dose of approximately 0.06 g/kg body weight of ^2H_2O (99.8 atom%, Cambridge Isotope Laboratories, MA, USA) and 0.14 g/kg body weight of $H_2^{18}O$ (10.0 atom%, Cambridge Isotope Laboratories, MA, USA) was orally given to each subject via a drinking straw. After the dose administration, the subjects refrained from eating and drinking over a 4-h equilibration period (4 h sampling) for measurement of total body water. The second voided urine in the morning of day 1 (the day after the DLW dose) and day 14 (at the same time as the voiding on day 1) were collected for measurement of the isotopic (^2H and ^{18}O) elimination rate.

The procedure for specimen analysis and for subsequent data analyses was described previously (Ishikawa-Takata *et al.*, 2007). Briefly, the isotopic analyses were conducted using the Isotope Ratio Mass Spectrometry (IRMS) *DELTA Plus* equipment (Thermo Electron Corporation, Bremen, Germany) and calibrated using Vienna Standard Mean Ocean Water (V-SMOW), 302B, and the Greenland Ice Sheet Precipitation (GISP) standard provided by the International Atomic Energy Agency. Each measurement of samples and the corresponding references was performed in duplicate. The average s.d. through the analyses were 0.5‰ for ^{2}H and 0.03‰ for ^{18}O.

TEE (kcal/day) calculation was performed using a modified Weir's formula Weir, 1949 based on rCO_2 (mol/day) and food quotient (FQ):

$$TEE = 3.9\times(rCO_2/FQ) + 1.1\times(rCO_2)$$

FQ was derived from the dietary assessment data (g/day) of DHQ using an equation of Black *et al.* (1986). The average value of all subjects (0.867) was used for all subjects to estimate TEE.

Assessment of other variables possibly related to the rEI

Lifestyle, behavioral and psychological variables possibly related to the rEI were obtained from the four-page questionnaire as follows: educational attainment, alcohol drinking, history of diet experiences, desire for body weight change, and difference between ideal and measured body weight.

A physical activity level was calculated as TEE divided by basal metabolic rate (BMR). BMR was estimated according to the 6th Recommended Dietary Allowances for Japanese Ministry of Health Welfare (1999).

Statistical analysis

We excluded 17 subjects who was non-Japanese ($n=1$), who was obese ($n=1$), who did not complete at least first or second DHQ ($n=2$), who had left more than 40 items blank in the questions regarding frequency for 121 selected food and beverage items in DHQ ($n=4$), who rEI outside the range of 3.0–16.0 MJ/day ($n=2$), or who did not provide sufficient urine sample volume ($n=7$). Thus, 140 subjects (67 men and 73 women) were included in the present analysis.

As we monitored the body weight change during the assessment period of rEI by second DHQ (rEI_{DHQ2}), we estimated EI (eEI) from TEE_{DLW} with a correction for change in body energy store during the survey period (Bathalon *et al.*, 2000):

$$eEI = TEE + (\Delta wt\times0.03)$$

where TEE is measured as MJ/day, Δwt is measured as g/day between visits 1 and 3, and 0.03 MJ/day (7 kcal/day) is the energy cost of weight change (Saltzman and Roberts, 1995). The eEI was used for the validation of rEI_{DHQ2}. In contrast, this correction of change in body energy store was not considered for the validation of rEI_{DHQ1} because of the lack of the monitoring.

The results were expressed as the mean and s.d. Mean differences between sexes and among methods were tested by the non-paired *t*-test and paired *t*-test, respectively. The Pearson and Spearman correlation coefficient was used to examine correlations between the test and the reference methods. Furthermore, the study participants were classified into tertiles of energy intake according to the distribution of

Table 1 Characteristics of 140 Japanese men and women aged 20–59 years included in the analyses[a]

	Men (n = 67)	*Women (n = 73)*
Age (years)	39.4±11.1	38.5±10.4
Body height (cm)	169.3±6.3	157.9±6.1[e]
Body weight (kg)	67.3±9.7	53.9±7.3[e]
BMI (kg/m^2)[b]	23.3±2.9	21.6±2.7[e]
<18.5	5 (7)	10 (14)[i]
18.5–24.9	39 (58)	55 (75)
⩾25.0	23 (34)	8 (11)
Educational attainment		
High school or less	28 (42)	23 (32)[h]
Technical or professional school	5 (7)	28 (38)
University or more	34 (51)	22 (30)
History of diet experience[c]		
No	58 (87)	57 (78)
Yes	9 (13)	16 (22)
Desire for weight change		
Reduction	37 (55)	50 (68)
No change	20 (30)	20 (27)
Increase	10 (15)	3 (4)
Difference between ideal and measured body weight (kg)[d]	−4.2±6.7	−4.5±4.3
Frequency of alcohol intake (times/week)	2.6±2.7	1.0±1.9[e]
Physical activity level	1.70±0.21	1.69±0.27
Body weight change during survey (g/day)	−23±55[j]	−2±45[g]
TEE_{DLW} (MJ/day)	10.7±1.7	8.3±1.2[e]
eEI_{DLW} (MJ/day)	10.0±2.1	8.2±2.0[e]
rEI_{DHQ1} (MJ/day)	8.8±2.4	7.7±1.7[f]
rEI_{DHQ2} (MJ/day)	8.9±2.5	7.4±1.5[e]

Abbreviations: BMI, body mass index; DHQ, diet history questionnaire; DHQ1, first measurement of DHQ before dose of DLW; DHQ2, second measurement of DHQ 2 weeks after dose of DLW; DLW, doubly labeled water method; eEI, estimated energy intake = TEE_{DLW} + (body weight change during survey × 0.03); rEI_{DHQ}, reported energy intake assessed with self-administered DHQ; TEE_{DLW}, total energy expenditure measured by DLW.

[a]Mean ± s.d. or *n* (%).

[b]The categorization was based on the Japan Society for the Study of Obesity (Matsuzawa *et al.*, 2000).

[c]Dieting was defined as at least 2 kg intentional reduction of body weight within 1 month.

[d]Ideal body weight was evaluated by the following question: how many kilograms is your ideal body weight? Difference between ideal and measured body weight was calculated, as ideal body weight (kg)—measured body weight (kg), to evaluate the degree of desire for body weight change.

[e–g]Difference between sexes by non-paired *t*-test: [e]$P<0.001$, [f]$P<0.01$, [g]$P<0.05$.

[h,i]Significant difference between sexes in all categories by χ^2 test: [h]$P<0.001$, [i]$P<0.01$

[j]Difference within sexes from 0 by paired *t*-test: $P<0.01$.

the test and the reference methods, and the proportions of subjects classified into the same, adjacent or opposite tertiles were determined.

To evaluate the prevalence of under- or over-reporters, we calculated 95% confidence limits of rEI_{DHQ1}/TEE_{DLW} and rEI_{DHQ2}/eEI_{DLW} as a cutoff value proposed by Livingstone and Black (2003). Then, subjects with rEI_{DHQ1}/TEE_{DLW} and rEI_{DHQ2}/eEI_{DLW} smaller than 0.84 or larger than 1.16 were considered as under- or over-reporters, respectively.

A stepwise multiple regression analysis was performed to evaluate the influence of sociodemographic, lifestyle, behavioral and psychological factors on rEI_{DHQ1}/TEE_{DLW} and rEI_{DHQ2}/eEI_{DLW}, simultaneously. The following potential factors were entered into the model as the independent variables: age, BMI, body height, residential area, educational attainment, physical activity level, frequency of alcohol drinking, desire for body weight change, difference between ideal and measured body weight, and history of diet experience.

To examine the reproducibility, we compared mean rEIs between first and second DHQs (DHQ1 and DHQ2, respectively). Furthermore, the Pearson correlation coefficients were used to compare the rEIs assessed with DHQ1 and DHQ2.

All statistical analyses were performed using version 8.2 of the SAS software package (SAS Institute Inc., Cary, NC, USA). The test was considered significant at a P-value of <0.05.

Results

Basic characteristics of the study subjects, the mean TEE_{DLW}, eEI, first and second measurements of rEI by the DHQ (rEI_{DHQ1} and rEI_{DHQ2}) are shown in Table 1. Men had the higher BMI than women (23.3 versus 21.6 kg/m^2, $P<0.001$). Twenty-three of 67 men and eight of 73 women were overweight (BMI $\geqslant 25$ kg/m^2). This table also shows body weight change during the TEE measurement, between visits 1 and 3. Mean body weight in men, although not in women, significantly changed by -23 ± 55 g/day ($P<0.01$ by paired t-test). Mean rEI_{DHQ1} was significantly lower than mean TEE_{DLW} by 1.9 ± 2.4 MJ/day (16.4%, $P<0.001$) for men and 0.6 ± 1.9 MJ/day (6.0%, $P<0.01$) for women. Mean rEI_{DHQ2} was also significantly lower than mean eEI_{DLW} by 1.1 ± 2.7 MJ/day (9.1%, $P<0.001$) for men and 0.8 ± 2.4 MJ/day (4.6%, $P<0.01$) for women.

Table 2 shows reporting accuracy of energy intake assessed with DHQ expressed as rEI_{DHQ1}/TEE_{DLW} and rEI_{DHQ2}/eEI_{DLW}. The rEI_{DHQ1}/TEE_{DLW} and rEI_{DHQ2}/eEI_{DLW} was 0.84 and 0.91 for men and 0.94 and 0.95 for women, respectively, resulting in a significantly lower rEI_{DHQ1}/TEE_{DLW} ratio for men than for women ($P<0.05$). There was a wide range in reporting accuracy of DHQ1; 31 and 51% were identified as acceptable, and 58 and 32% as under-, and 10 and 18% as over-reporters for men and women, respectively.

The rEI_{DHQ1} and TEE_{DLW} were significantly correlated only for men (Pearson correlation coefficient = 0.34, Spearman correlation coefficient = 0.33), but not for women (0.22 and 0.16, respectively). Forty-one, 45 and 14% of the subjects were cross-classified into the same, the adjacent and the opposite tertiles of the respective distributions of rEI_{DHQ1} and TEE_{DLW}, respectively (Figure 1a). The results of the correlation between rEI_{DHQ2} and eEI_{DLW} were similar (Figure 1b).

Table 3 shows the results of multiple regression analysis with rEI_{DHQ1}/TEE_{DLW} and rEI_{DHQ2}/eEI_{DLW}, as the dependent variables to examine the prediction of accuracy of reporting energy intake. For men, frequency of drinking alcohol, the difference between ideal and measured body weight, and history of diet experience correlated significantly and

Table 2 Reporting accuracy of energy intake determined by the self-administered diet history questionnaire[a]

	DHQ1			DHQ2		
	All (n = 140)	*Men (n = 67)*	*Women (n = 73)*	*All (n = 140)*	*Men (n = 67)*	*Women (n = 73)*
Reporting accuracy[b]	0.89 ± 0.22	0.84 ± 0.21	0.94 ± 0.22[c]	0.93 ± 0.30	0.91 ± 0.26	0.95 ± 0.33
Underreporters (*n* (%))	62 (44)	39 (58)	23 (32)[d]	64 (46)	30 (45)	34 (47)
Acceptable reporters (*n* (%))	58 (41)	21 (31)	37(51)	48 (34)	27 (40)	21 (29)
Overreporters (*n* (%))	20 (14)	7 (10)	13 (18)	28 (20)	10 (15)	18 (25)
Pearson's correlation coefficient	0.40[e]	0.34[f]	0.22	0.36[e]	0.35[f]	0.11
Spearman correlation coefficient	0.35[e]	0.33[f]	0.16	0.36[e]	0.41[e]	0.07

Abbreviations: DHQ1, first measurement of DHQ before dose of DLW; DHQ2, second measurement of DHQ 2 weeks after dose of DLW; DLW, doubly labeled water; eEI, estimated EI.

[a]Mean ± s.d. or *n* (%).

[b]Reporting accuracy was assessed as the ratio of energy intake to total energy expenditure (rEI_{DHQ1}/TEE_{DLW}) and the ratio of energy intake to estimated energy intake (rEI_{DHQ2}/eEI_{DLW}), respectively. eEI was determined by using a correction for change in body energy during the measurement period, as TEE ± (body weight change during survey × 0.03). Under-, acceptable, and over-reporters were defined as the ratio rEI_{DHQ}/TEE_{DLW} and rEI_{DHQ}/eEI_{DLW} <0.84, 0.84–1.16 and >1.16, respectively.

[c]Difference between sex by non-paired t-test: $P<0.01$.

[d]Significant difference between sexes in all categories by χ^2 test: $P<0.01$.

[e,f]Correlation coefficients between two methods: [e]$P<0.001$, [f]$P<0.01$.

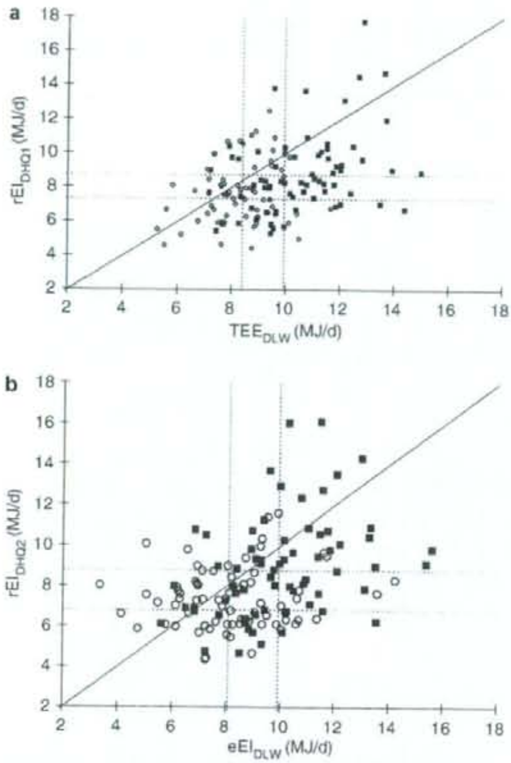

Figure 1 (**a**) Comparison of the first measurement of energy intake determined by the self-administered diet history questionnaire (rEI_{DHQ1}) with total energy expenditure measured by the doubly labeled water method (TEE_{DLW}) (■ = 67 men, ○ = 73 women). The dotted lines divide intake according to the tertiles of distribution. A straight line is $y=x$. Pearson and Spearman correlation coefficient was 0.40 and 0.35, respectively (both $P<0.001$). (**b**) Comparison of the second measurement of energy intake determined by the self-administered diet history questionnaire (rEI_{DHQ2}) with estimated energy intake (eEI_{DLW}) determined by a correction of body weight change during survey period, as TEE + (Δwt × 0.03), (■ = 67 men, ○ = 73 women). The dotted lines divide intake according to the tertiles of distribution. A straight line is $y=x$. Pearson and Spearman correlation coefficient was both 0.36 ($P<0.001$).

positively, and physical activity level negatively with rEI_{DHQ1}/TEE_{DLW}. For women, age and educational attainment correlated significantly and positively, and BMI negatively with rEI_{DHQ1}/TEE_{DLW}. We also conducted the same analysis with rEI_{DHQ2}/eEI_{DLW}. Body height, BMI and physical activity level significantly and negatively correlated with rEI_{DHQ2}/eEI_{DLW} for women. On the other hand, no factors attained the significance level for men.

The Pearson correlation coefficients between rEI_{DHQ1} and TEE_{DLW} slightly improved in both sexes after adjustment for the above-mentioned related factors (0.42 for men and 0.37 for women).

We also examined reproducibility of energy intake between DHQ1 and DHQ2. The rEI_{DHQ2} was significantly lower than rEI_{DHQ1} for women (the difference was -0.3 ± 1.1 MJ/day, $P=0.03$), but not for men. The Pearson correlation coefficient between rEI_{DHQ1} and rEI_{DHQ2} was 0.79 for men and 0.76 for women.

Discussion

To our knowledge, this is the first report in a non-Western country to validate energy intake estimated with a dietary assessment questionnaire against TEE measured by DLW method. Moreover, the sample size was relatively large compared to the previous studies with the same purpose and method (Sawaya *et al.*, 1996; Kroke *et al.*, 1999; Andersen *et al.*, 2003).

The mean rEI_{DHQ1} was 11.0% less (16.4% for men and 6.0% for women) than the mean TEE_{DLW}. Several validation studies have shown that dietary assessment instruments underestimated daily energy intake (Livingstone *et al.*, 1990; Hill and Davis, 2001). The degree of such error, under- or overestimation, has also been examined using TEE measured by the DLW method (Sawaya *et al.*, 1996; Kroke *et al.*, 1999; Andersen *et al.*, 2003; Livingstone and Black, 2003). Average underreporting in the previous studies between EI from dietary assessment questionnaires and TEE measured by DLW ranged from 10 to 38% (Sawaya *et al.*, 1996; Subar *et al.*, 2003), which depends on sample size and subjects (Trabulsi and Schoeller, 2001).

For the individual ranking, the rEI_{DHQ1} significantly and positively correlated with TEE_{DLW} ($r=0.40$, $P<0.001$), showing a correlation similar to or relatively higher than those observed in the previous studies ($r=0.06$–0.48) (Kroke *et al.*, 1999; Bathalon *et al.*, 2000). Acceptable reporting was observed in 41% of the subjects, whereas 44% underreported and 14% over-reported. Underreporting of energy intake therefore seems to be a more serious problem than over-reporting.

In this study, the mean rEI_{DHQ1}/TEE_{DLW} ratio was significantly lower in men than in women. Further, the rate of underreporting was higher in men than in women. In a previous analysis of individual data from 21 studies, in contrast, the proportion of underreporters did not statistically differ between sexes (Black, 2000). In our previous study using semi-weighed diet records in 4 days × 4 seasons, the mean value of the ratio of rEI to BMR estimated from sex, age and body weight was not statistically different between sexes (Okubo *et al.*, 2006). In the DHQ, the portion sizes of food items are standardized regardless of sex, for example as 'one small cup'. The subjects then select the relative portion size from the five categories given except for rice, bread, noodles, other wheat foods and miso soup. This structure

Table 3 Result of multiple regression analysis by stepwise procedure with the ratio of energy intake to total energy expenditure ($rEID_{HQ1}/TEE_{DLW}$ and rEI_{DHQ2}/eEI_{DLW}) as dependent variables[a]

Independent variable[b]	Men (n = 67)						Women (n = 73)					
	DHQ1			DHQ2			DHQ1			DHQ2		
	Partial regression coefficient[c]	s.e.[d]	P-value	Partial regression coefficient[c]	s.e.[d]	P-value	Partial regression coefficient[c]	s.e.[d]	P-value	Partial regression coefficient[c]	s.e.[d]	P-value
Age (years)	—	—	—	—	—	—	0.005	0.002	0.04	—	—	—
BMI (kg/m^2)	—	—	—	—	—	—	−0.036	0.009	<0.001	−0.049	0.015	<0.01
Body height (cm)	—	—	—	—	—	—	—	—	—	−0.016	0.006	0.02
Residential area	—	—	—	—	—	—	—	—	—	—	—	—
Educational attainment, (more than University versus high school or less as reference)	—	—	—	—	—	—	0.145	0.053	<0.01	—	—	—
Physical activity level	−0.356	0.120	<0.01	—	—	—	—	—	—	−0.480	0.154	<0.01
Frequency of drinking alcohol (times/week)	0.026	0.009	<0.01	—	—	—	—	—	—	—	—	—
Desire for body weight change	—	—	—	—	—	—	—	—	—	—	—	—
Difference between ideal and measured body weight (kg)	0.013	0.003	<0.01	—	—	—	—	—	—	—	—	—
History of diet experience (yes versus no as reference)	0.170	0.071	0.02	—	—	—	—	—	—	—	—	—

[a]TEE_{DLW}, total energy expenditure measured by doubly labeled water method (DLW); rEI_{DHQ}, reported energy intake assessed with self-administered diet history questionnaire (DHQ); DHQ1, first measurement of DHQ before dose of DLW; DHQ2, second measurement of DHQ 2 weeks after dose of DLW. Reporting accuracy were assessed as the ratio of energy intake to total energy expenditure (rEI_{DHQ1}/TEE_{DLW}) and the ratio of energy intake to estimated energy intake (rEI_{DHQ2}/eEI_{DLW}), respectively. Estimated EI (eEI) was determined by using a correction for change in body energy during the measurement period, as TEE + (body weight change during survey × 0.03).

[b]See table 1 for the definition of each independent variable. Age (as a continuous variable), BMI (as a continuous variable), body height (as a continuous variable), residential area (Hokuriku, Shikoku, North Kyushu, and South Kyushu), educational attainment (high school or less, technical or professional school, or university or more), physical activity level (as a continuous variable), frequency of alcohol drinking (as a continuous variable), desire for body weight change (reduction, no change or increase), difference between ideal and measured body weight (as a continuous variable), history of diet experience (yes or no).

[c]Partial regression coefficient; change in dependent variable related to a 1-U change in independent variable.

[d]Standard error (s.e.) of the regression coefficient.

might have led to relative over- and underreporting of energy in women and men, respectively.

The rEI_{DHQ1}/TEE_{DLW} was significantly and independently correlated with several anthropometric and behavioral factors (Table 3). Several previous studies have already examined non-dietary factors, such as physiological (Zhang et al., 2000; Livingstone and Black, 2003) and psychological (Johansson et al., 1998; Bathalon et al., 2000; Tooze et al., 2004) factors associated with reporting accuracy of energy intake. After adjusting for these variables, the validity slightly improved (Pearson correlation coefficient was 0.42 for men and 0.37 for women). Therefore, these non-dietary factors are needed to consider when evaluating rEI.

This study has several limitations. First, FQ was derived from dietary assessment data by DHQ. Therefore, TEE was not theoretically independent of EI. Second, the surveyed period for the first measurement of EI by DHQ (DHQ1) was ahead of, and not overlapping with, TEE measurement by the DLW method. Third, we used the TEE as gold standard for the validation of DHQ1 without any consideration for a possible body weight change during the assessment period because of lack of the data. Fourth, we used the TEE with a correction for change in body weight during the survey period as gold standard for the validation of DHQ2, because the body weight has significantly changed in men. Fifth, the change in body composition, such as change in fat mass and fat-free mass, is probably the better indicator than the change in body weight for the correction of energy content for the study purpose. Sixth, the rEI_{DHQ1} was significantly lower than the rEI_{DHQ2} for women. Intentional or non-intentional intervention effect might have influenced dietary behaviors between the first and the second measurement. As shown in Table 3, the factors affecting reporting accuracy of energy intake were different between the two measurements. This may be one of the reasons. Seventh, we applied a two-point rather than multipoint method for the measurement of TEE_{DLW}. Eighth, the subjects were not randomly sampled from the general Japanese population. Moreover, the survey areas were not equally distributed over the country but were rather selected mostly from the Western parts of Japan.

In summary, the energy intake assessed with DHQ correlated low to modestly with TEE measured by DLW. In addition, DHQ underestimated energy intake at a group level. Caution is needed when energy intake was evaluated by DHQ at both individual and group levels.

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

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Abstract

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

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

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

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

Background

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

Cardiovascular fitness is usually evaluated as the maximal oxygen uptake per body mass (Vo_2max, $mL \cdot kg^{-1} \cdot min^{-1}$). The Japanese Ministry of Health Labour and Welfare in 2006 proposed Vo_2max reference values for each age group to prevent LSRD [9]. These Vo_2max reference values were determined by the "Committee for the Determination of the Recommended Exercise Allowance and Exercise Guide" established in August 2005, and were referenced in the "Exercise and Physical Activity Reference Quantity for Health Promotion 2006 (EPAR2006)". Originally, the "Recommended Quantity of Exercise for Health Promotion (1989)" had been formulated to mainly target the prevention of coronary artery disease. With the passage of more than 15 years following the establishment of this standard, the morbidity pattern of people has worsened and LSRD have increased in prevalence. In order to face this situation, the EPAR2006 was made based on the latest scientific evidence, and was designed to maintain and promote the health of people and prevent LSRD by improving their capacity for physical activity and exercise. These Vo_2max reference values proposed in the EPAR2006 were determined by experts through the systematic review of literature regarding the relationship between Vo_2max and LSRD such as obesity, hypertension, hypercholesterolemia, diabetes, cerebrovascular disease, CVD mortality and all-cause mortality.

It is well known that Vo_2max decreases with age [10-20]. It has been suggested that the age-related decline in Vo_2max is a consequence of attenuation of central and peripheral functions such as stroke volume, heart rate max (HR_{max}), peripheral O_2 extraction, and lean body mass (LBM) or muscle mass [19,21-25]. Among these determinants, reductions in HR_{max} and LBM or muscle mass have been suggested to be primary factors [26,27]. While many studies on cardiovascular fitness have focused on cardiac measurements, it should be emphasized that muscle mass is one of the critical determinants of Vo_2max [13,14,19,24,26,28-30] since the amount of tissue available to extract oxygen during maximal exercise, i.e., muscle, can directly contribute to the value of Vo_2max. For example, Sanada et al. reported the MRI-measured lower body skeletal muscle mass was closely associated to the absolute Vo_2max during running [28,30]. Additionally, the age-related decrement in Vo_2max can be related to the age-associated muscle loss [24,19]. Further, it is important to notice that LBM or muscle mass can be maintained to some degree by exercise training, while such training cannot prevent age-related declines in HR_{max}, [26,27].

Therefore, we hypothesized that a certain level of muscle mass required to maintain sufficient cardiovascular fitness is present and that it could be a limiting factor of age-related Vo_2max attenuation. Based on this hypothesis, it is advantageous to Japanese women's health to propose such muscle mass required to maintain sufficient Vo_2max. Thus, the purpose of this study was to determine a required value of muscle mass to maintain the Vo_2max reference value determined by the Japanese Ministry of Health Labour and Welfare in 2006 (Ministry of Health, Labour and Welfare of Japan 2006).

Methods

Subjects

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

Percentage of muscle mass

The lean soft tissue mass of legs and arms were measured with a whole-body Dual Energy X-ray Absorptiometry (DXA) scanner (Hologic QDR-4500, Hologic INC., Waltham, MA, USA). The body regions were delineated according to specific anatomical landmarks using manual DXA analysis software (version11.2.3). The appendicular lean soft tissue mass was calculated as a sum of the lean soft tissue mass of the legs and the arms. The lean soft tissue mass of extremities assessed using DXA was assumed to represent appendicular skeletal muscle mass along with a small and relatively constant amount of skin and underlying connective tissues. The percentage of muscle mass (%MM) was calculated as follows;

%MM (%) = (Appendicular lean soft tissue mass)/Body weight × 100.

Vo_{2max}

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

Vo_{2max} reference values

The Japanese Ministry of Health Labour and Welfare proposed Vo_2max reference values to prevent life-style related illness for women [9]. The Vo_{2max} reference values are provided for each age group. The procedure to determine Vo_{2max} reference values was described in the EPARQ2006 [9]. In brief, these Vo_{2max} reference values were determined by experts through a systematic review of literature. The target age was 6 years and older. The target LSRD were obesity, hypertension, hyperlipemia, diabetes mellitus, cerebrovascular disorders, death due to circulatory diseases, osteoporosis, ADL and total mortality. By means of this systematic review, the threshold values of the Vo_{2max} or Vo_{2peak} at which the morbidity of LSRD statistically increases in each age group were collected from the literature. The average values of these threshold values for each age group were then calculated and designated as the Vo_{2max} reference values for preventing LSRD. The identified Vo_{2max} reference values ($mL \cdot kg^{-1} \cdot min^{-1}$) were 33 (20–29 yr), 32 (30–39 yr), 31 (40–49 yr), 29 (50–59 yr), and 28 (60–69 yr).

Analyses

First, a single regression analysis was used to test the correlation between age and Vo_2max, and between %MM and Vo_2max in V-group. Then, a multiple regression analysis was performed using Vo_2max as a dependent variable, and age and %MM as the independent variables. This analysis was based on the hypothesis that Vo_2max can be accounted for by age and %MM. In this hypothesis, we assumed that the age factor included Vo_2max determinant factors related to aging except for muscle mass, such as HR_{max}, maximal stroke volume, and peripheral O_2 extraction [21-23,25,27]. The validity of the prediction by the obtained regression equation was tested by applying the obtained regression equation to the CV-group. After the equation was cross-validated, the data from the two groups were pooled together to obtain the final prediction equation and in the subsequent analysis.

The purpose of the final prediction equation was to obtain the required %MM to maintain the reference Vo_2max value in each age group. Thus, the required %MM for each subject was recalculated by assigning the Vo_2max reference values and age in the final prediction equation. If the difference of the required %MM among the age groups was very small, the mean value of the required %MM was calculated to be used in the following analysis. To test the validity of the required %MM, the correlation between the sufficiency of Vo_2max, i.e., individual's Vo_2max as the percentage of the Vo_2max reference values (% Vo_2max reference values), and the sufficiency of the required %MM, i.e., individual's %MM as the percentage of the required %MM (%required-%MM), were tested.

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

Results

Physiological characteristics

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

Relationship between age and Vo_2max in V-group

Vo_2max in V-group was from 16.4 to 56.9 $ml.kg^{-1}min^{-1}$ (mean 33.5 ± 7.9) (Table 1). As expected, a strong nega-

Table 1: Characteristics of validation and cross-validation group

	V-group	CV-group
n	202	201
Age (yr)	41.4 ± 16.7	41.6 ± 16.9
Height (cm)	158.5 ± 6.4	157.9 ± 6.1
Body weight (kg)	54.4 ± 7.4	53.9 ± 7.3
Body mass index (kg/m^2)	21.6 ± 2.7	21.7 ± 2.9
Appendicular muscle mass (kg)	16.4 ± 2.4	16.1 ± 2.3
% MMI (%)	30.3 ± 3.2	30.0 ± 3.4
Vo_2max ($ml \cdot kg^{-1} \cdot min^{-1}$)	33.5 ± 7.9	32.7 ± 7.7

mean ± SD, V-group, Validation group; CV-group, Cross-validation group; %MM, percentage of muscle mass tive linear correlation was found between Vo_2max and age (Figure 1). The decrement was 2.58 ml.kg^{-1}min^{-1} per decade. The Vo_2max reference values for each age group in the EPAR2006 were superimposed in Figure 1. With increasing age, the proportion of subjects with Vo_2max values below the reference Vo_2max values increased.

Relationship between Vo_2max and %MM in V-group
%MM in V-group was from 18.7 to 37.3% (mean 30.3 ± 3.2%) (Table 1). There was also a strong correlation between Vo_2max and %MM, while the correlation was positive (Figure 2).

Multiple-regression analysis in V-group
Multiple regression analysis in V-group revealed that age ($R^2 = 0.286$) and %MM ($R^2 = 0.540$) were significant ($p < 0.0001$) contributors to the prediction of the measured Vo_2max. The multiple regression equation obtained in the V-group was the following: $Vo_2max = -0.135 \times Age + 1.315 \times \%MM - 0.799$. In this equation, R^2 and SEE were 0.522 and 5.4 mL · kg^{-1} · min^{-1}, respectively.

Cross-validation of the multiple regression equation
The multiple regression equation derived from the V-group was used to predict Vo_2max in the CV-group. Figure 3 shows the residual plot. There was not statistically significant correlation between the predicted Vo_2max and residual error ($p > 0.05$). Thus, the residual plot indicates that there was no bias in the prediction of Vo_2max of the CV-group using the multiple regression obtained in the V-group.

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

$$Vo_2max = -0.131 \times Age + 1.344 \times \%MM - 2.035. \quad (1)$$

In the final equation, analysis revealed that age ($R^2 = 0.282$) and %MM ($R^2 = 0.570$) were significant ($p < 0.0001$) independent contributors to the prediction of the measured Vo_2max. Figure 4 shows the residual plot of the multiple-regression. There was no statistically significant correlation between the predicted Vo_2max and residual error ($p > 0.05$). Thus, the residual plot indicates that there was no bias in the prediction of Vo_2max.

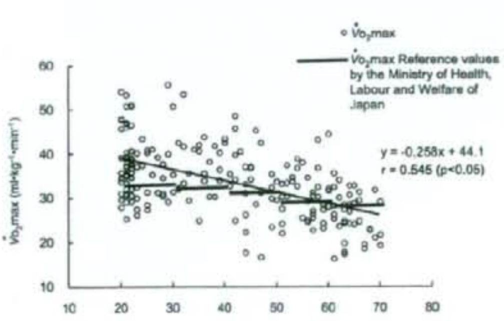

Figure 1
The relationship between age and Vo_2max in the V-group. The Vo_2max reference values by the Japanease Ministry of Health Labour and Welfare were shown for reference.

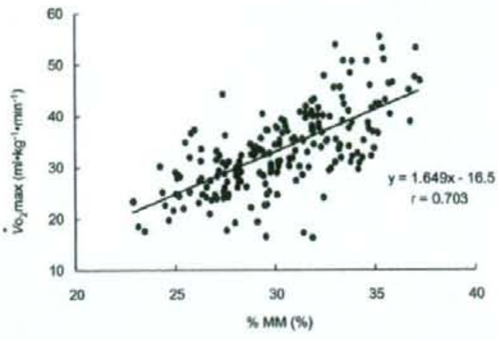

Figure 2
Relationship between percentage of muscle mass (%MM) and Vo_2max in the V-group.

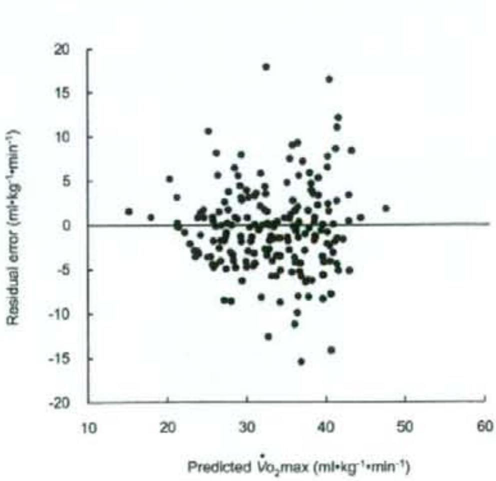

Figure 3
Relationship between estimated Vo_2max by the multiple regression equation and the residuals for the CV-group.

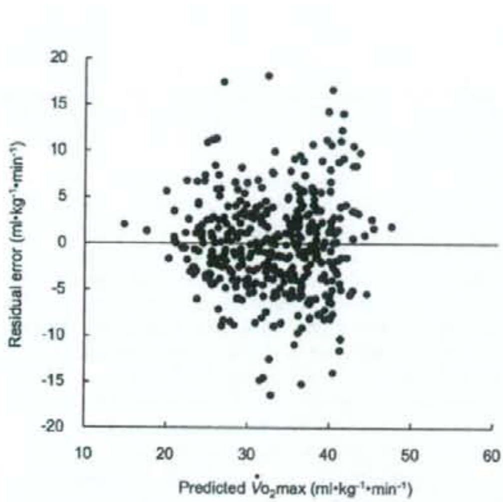

Figure 4
Relationship between estimated Vo_2max by the multiple regression equation and the residuals for both the V-group and the CV-group.

Estimation of the required %MM

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

$$\%MM = (0.131 \times Age + 2.035 + Vo_2max)/1.344. \quad (2)$$

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

The validity of the required %MM

Figure 6 shows the relationship between %Vo_2max reference values and %required-%MM. The %Vo_2max reference values positively correlated with %required-%MM ($r = 0.651$, $p < 0.05$).

Discussion

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

Required muscle mass

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

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

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

Mean ± SD; %MMI, percentage of muscle mass

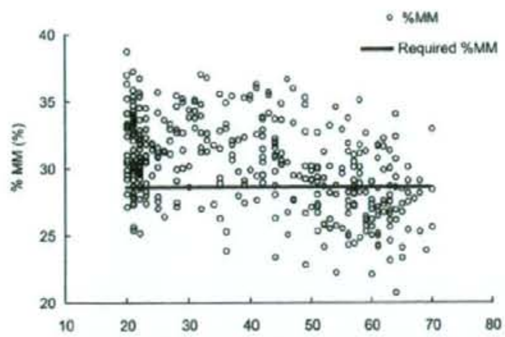

Figure 5
The relationship between age and the percentage of muscle mass (%MM) in the V-group and the CV-group. Required %MM is shown for reference.

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

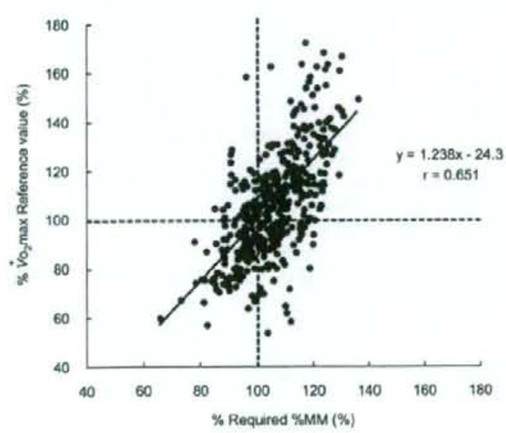

Figure 6
The relationship between the sufficiency of Vo_2max (%Vo_2max reference values) and the sufficiency of the required %MM (% required %MM) in both the V-group and the CV-group. Solid line: regression line, dashed line: lines of 100% of Required %MM and 100% of Vo_2max reference values.

of the age-related decline in Vo_2max or to increase Vo_2max, it would be advisable to recommend some form of strength training as well as aerobic training especially for individuals who do not achieve the required %MM.

Several prior studies demonstrated the significance of fat free mass, muscle mass, and/or muscle function to morbidity and mortality, although there are few researches targeting women [31-33]. The Japanese Ministry of Health Labour and Welfare also has admitted the importance of muscle mass and muscle function to prevent LSRD and/or mortality in EPAR2006. However practical target values have not been offered in the statement due to the lack of evidences compared to Vo_2max. In this present study we determined the target value of muscle mass through the Vo_2max reference values, which already has strong evidences. Although we have not confirmed the direct relation between muscle mass and LSRD morbidity and/or mortality, we believe Japanese women could aim to achieve the required %MM as one of targets for their health. Whether an increase of skeletal muscle mass would result in an improvement of exercise capacity and or reduce morbidity and mortality needs to be confirmed by future studies.

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

Prediction of Vo2max from age and muscle mass

The residuals of the multiple regression might be due to the approximation that all age-related determinant factors were included in age in the multiple regression. In the present model, we hypothesized that determinants such as HR_{max}, maximal stroke volume, and peripheral O_2 extraction were age-related, and therefore their effects were included in the factor of age. It was suggested that HR_{max} [14,22,26,29,34-39] and peripheral O_2 extraction [21,34] do decline with age, and are not influenced by exercise training. However, although maximum stroke volume was also suggested to decline with age in sedentary individuals [23], it was suggested that age-related decline of maximum stroke volume was prevented by exercise [21,34]. Thus, the simplification must be the error factor, and it is likely in future to improve the multiple regression equations using these age-related Vo_2max determinants, and to improve the estimation of the required MMI.

We studied only a statistical relationship between Vo_2max and muscle mass. Therefore, the results do not necessarily suggest a cause-effect relationship. It is possible that muscle mass and Vo_2max are physiologically unrelated but indirectly correlated, i.e., people with a high Vo_2max may be more physically active and perform activities that increase muscle mass. However, muscle mass is highly likely physiologically important determinant of Vo_2max because the amount of tissue available to extract oxygen during maximal exercise directly contribute to the value of Vo_2max.

Study limitations

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

Conclusion

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

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

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

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