福岡大学博士学位論文

アスリートにおける短期間の減量と増量が身体組成とエネルギー代謝におよぼす影響

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学位申請論文

要旨

【目的】

　一般の減量とは異なる目的を持ち，特殊な方法で行う減量方法がある．これは急速減量と呼ばれ，日々激しいトレーニングを行っており，体脂肪が低値にも関わらず試合に向けた競技アスリートが行う減量方法である p．これら競技アスリートはその後数時間から１日にかけて体重を増加させる急速増量を行う．

　多くの研究では対象者であるアスリートへの負担を軽減させるため，二次間接法である生体電気インピーダンス法や皮脂厚法を用いて，身体組成の変化を調査しており，除脂肪量（fat free mass; FFM）の多くが減少すると報告している．急速減量には飲水制限が伴うため，体水分量（total body water; TBW）の急激な減少により脱水状態が引き起こされやすくなる可能性があり，急激な変化がある場合に正しく身体組成の変化を反映しているかは不明である．したがって，体重階級制アスリートの急速減量中の TBW の変化を考慮した身体組成の評価が必要である．

　また，エネルギー代謝の評価がアスリートのパフォーマンスに必要である一つの理由は，エネルギー出納の結果が体重および身体組成の変化に表れるからである．エネルギー保存の法則は，エネルギー摂取 (energy intake; EI) とエネルギー消費 (energy expenditure; EE) との関係が体重や身体組成変化も含めた系において厳密に成り立っている．水分の変動がな

ければ，単純に EI が総エネルギー消費量(total energy expenditure; TEE)を上回れば体重は増加し，逆になれば体重は減少する．しかし，水分の変動があった場合にはこの限りではないので，TBW の評価はエネルギー出納を考える上で非常に重要である．同時に，決められた期間でウェイトコントロールを行うには EI と EE の評価が不可欠となる．

　そこで，本研究の目的は 1) 短期間の急速減量と急速増量を行う競技アスリートの身体組成の変化を明らかにする，2) 短期間の急速減量と急速増量を行う競技アスリートのエネルギー代謝の変化を明らかにすることとした．

　これらの目的を達成する為に，研究 1（第二章）では異なる 2 種類の安定同位体を用いた短期間の身体組成評価のモデル作成および検討を行い，研究 2（第三章）では急速減量と急速増量が身体組成とエネルギー代謝に与える影響の検討を行った．

【方法】

研究 1

　若年男性 10 名（年齢; 23.1±1.6 歳, 身長; 171.7±3.6 cm, 体重; 63.6±4.5 kg, BMI; 21.6±1.3 kg/m^2）を対象とした．対象者には 1 回目と 2 回目（Baseline^{1st}; BL^{1st}, Baseline^{2nd}; BL^{2nd}）の身体組成測定の間，体重の変動がないように依頼した．1 回目と 2 回目の安定同位体投与は 3 日間の間隔をおいた．TBW 測定のための安定同位体は 1 回目と 2 回目で異なる同位体を使用した．

　形態測定は 12 時間以上の絶食の状態，排尿後に行った．身長は 0.1 cm 単位で測定できる身長計で測定した．体重は軽装の下着のみでデジタル体重計（Shinko Denshi Vibra Co. Ltd, Tokyo, Japan）を使用し 0.01 kg まで測定した．水中体重の測定と同時に 100%酸素の再呼吸法で残気量を推定し，補正した水中体重により身体密度を算出した．TBW は重水素水（^{2}H$_2$O 99.8 atom%; Taiyo Nippon Sanso, Tokyo, Japan）と重酸素水（H$_2$^{18}O 10.0 atom%; Taiyo Nippon Sanso, Tokyo, Japan）の安定同位体を用いて推定した．

　%Fat は Siri の 3 成分モデルの式を用いて算出した．算出された%Fat より FM を算出し，体重より FM と TBW を引いた値を除脂肪固形分量(free fat dry solid ; FFDS)，TBW と FFDS を足した値を FFM として算出した．

研究 2

　体重階級制競技経験(ボクシング，柔道)のある若年男性 10 名(年齢 20 ± 1.4 歳)を本研究の

対象とした.

　本研究は，急速減量前の通常体重時(通常期, Baseline; BL)，急速減量後の減量時体重(減量

期, Wegiht loss; WL)，急速増量の増量時体重(増量期, Weght regain; WR)で 3 回の測定を順に

行った.対象者には急速減量前の通常期の体重より 1 週間で体重の 5%を目標に減量を行い,

その後 1 日で体重を減量前の体重に戻してもらうように指示した.脱水法（サウナ，飲水

制限），食事制限, 活動量の増大による減量が考えられるが, 減量方法は対象者に一任した.

身体組成の測定はメタボリックチャンバーに入室する日の早朝に 12 時間以上の絶食の状態,

排尿排便後に行った.身長は 0.1cm 単位で測定できる身長計で測定された.体重は軽装で

デジタル体重計(Shinko Denshi Vibra Co. Ltd, Tokyo, Japan)を使用し 0.01kg まで測定された.

水中体重の測定と同時に 100%酸素で再呼吸を行い，残気量を推定し，補正した水中体重に

より身体密度を測定した.TBW は重水素(^{2}H$_2$O 99.8 atom%; Taiyo Nippon Sanso, Tokyo, Japan)

と重酸素(H$_2$^{18}O 10.0 atom%; Taiyo Nippon Sanso, Tokyo, Japan)の安定同位体を用いて推定し

た.

　%Fat は Siri の 3 成分モデルの式を用いて算出した.算出された%Fat より体脂肪量を算出

し，体重より FM と TBW を引いた値を FFDS，TBW と FFDS を足した値を FFM として算出した．

　対象者は測定の前日から運動後余剰熱産生のエネルギー消費量への影響を避けるために激しい運動を行わないように指示された．カロリーメータ内では座位安静とし，主な時間は読書，テレビ観賞，書き物をして過ごした．対象者は起きた状態を維持し，睡眠時間以外は寝ないように監視された．測定日の夕方(17:00)から対象者はメタボリックチャンバーに入室した．17:40 に対象者は各期間に合わせ，各自で持ってきた自由食を摂取し始め，18:00 に食べ終わるようにした．23:00 に就寝し 6:00 に起床した．この間を睡眠時代謝（sleeping metabolic rate; SMR）とした．6:00 に起床してからは基礎代謝（basal metabolic rate; BMR）を測定するために仰臥位で 6:30 まで過ごしてもらった．その間に対象者が寝ないように監視した．その後，6:40 に朝食を食べ始め 7:00 丁度に朝食を食べ終え，その後 3 時間座位安静を保ち，10:30 にメタボリックチャンバーから退出した．

【結果】

研究 1

　3 成分モデルで評価された身体組成は，各項目において，有意な変化を認めなかった（体重; －0.2 ± 0.5 kg, p = 0.17, %Fat; －0.1 ± 0.5 %, p = 0.49, 体脂肪量; －0.1 ± 0.4 kg, p = 0.36, 体水分量; －0.1 ± 0.4 kg, p = 0.56, 除脂肪固形分量; 0.0 ± 0.4 kg, p = 0.71）．変動係数は，いずれの項目において 3%未満であった．

研究 2

　体重は通常期から減量期にかけて有意に減少し (-6.0 ± 0.9%)，その後の増量期に増加した．しかしながら，増量期の体重は通常期の体重まで増加しなかった．TBW は通常期から減量期にかけて有意に減少し (-5.1 ± 1.8%)，その後の増量期に増加した．FFDS は通常期に対し減量期に有意に減少したが (-3.9 ± 1.5%)，その後の増量期に増加した．FM は通常期から減量期にかけて有意に減少し (-15.5 ± 12.1%)，増量期で増加したが， 通常期の FM に比べ有意に低値であった(-10.0 ± 6.5%)．急速減量の体重減少に寄与した FM と FFM の割合として，FM が 33.8 ± 13.6%，FFM が 66.2 ± 13.6%であった．急速増量に寄与していた FM と FFM の割合は FM が 9.2 ± 32.3%，FFM が 90.8 ± 32.3%であった．

　1 日当たりに換算した BMR は通常期に対して減量期で減少する傾向にあったが(P=0.09)，

増量期では通常期と有意な変化は見られなかった．また，体重と FFM で補正した BMR は

すべての期間において有意な変化は見られなかった．

SMR は通常期と比較したときに減量期で有意な減少を示したが,体重増加に伴い通常期ま

で増加した．体重と FFM で補正した SMR は通常期に比べ減量期では有意な減少を示した

が，その後の急速増量で増加した．

【考察】

　本研究 1 の目的は異なる２種類の安定同位体（重水素水と重酸素水）を用いた短期間の

TBW 推定法および３成分モデルの検討であった．結果として，体重及び身体組成の有意な

変化を認めなかった．各測定の変動係数は，0.5-2.2%を示し，最も大きい変動係数は体脂肪

量と%fat であった．身体組成の精度検定を行った研究によると，再テスト法による変動係

数 2.3±1.9%と報告され．本実験での変動係数は，先行研究の値の範囲内であり，信頼性の

ある値として考えられる．体重階級制アスリートの体重変動は減量前体重の５％以上減少し，

脱水は 2%以上起こることが予想される．１週間の短期間で異なった安定同位体を用いた

TBW の測定で身体組成を評価した場合も結果が示すように，３成分（体脂肪量, 体水分量,

除脂肪固形分量）を精度よく評価することが可能であると考えられる．

　研究１の結果を基に研究２を実施した．研究２の主な知見は体重階級制のアスリートが行

う典型的な急速減量とその後１日の増量法による体重の増減には主に TBW と FM が寄与し

ていることである．本研究では体重階級制アスリートが行う急速減量と急速増量の身体組

成の変化，特に FM:FFM の減少の比率と TBW の変化に着目し検討を行った．その結果，減

量前と比べて急速減量後に体重が 6%減少し，減量後の増量で体重が 4.6%増加した．体重減

少の 33.8%が FM で 66.2%が FFM であった．体重増加の 9.2%が FM で 90.8%が FFM であっ

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た. 3 成分モデルで示すと急速減量による体重の変化の 50.6%が TBW であり，33.8%が FM，15.6%が FFDS の減少であった．減量後の増量では体重増量の 67.5%が TBW であり，9.2%が FM，23.3%が FFDS の増量であった．本研究において，TBW が減量後に大幅に減少し，増量後に増加したことを考えると，TBW の変化にはグリコーゲンの変化が寄与しており，FFDS の減少はグリコーゲンの減少ではないかと推察される．本研究のアスリートは短期間で FM:FFM が約 1:2 とより多くの FFM が減少しているが，FFM の減少のうちの大部分が TBW であったことから，本研究では先行研究と異なり，BMR が急速減量($P=0.09$)や急速増量により影響を与えなかった可能性が考えられる．また，本研究では TBW 測定のゴールデンスタンダードである安定同位体希釈法を用いたが， TBW の増減に BMR の変化が伴わなかったことから，TBW の増減は BMR に影響を与えないかもしれない．

第一章

研究の背景

I-I. 急速減量と急速増量

一般の減量とは異なる目的を持ち，特殊な方法で行う減量方法がある．これは急速減量と呼ばれ，日々激しいトレーニングを行っており，体脂肪が低値にも関わらず試合に向けた競技アスリートが行う減量方法である．これら競技アスリートはその後数時間から１日にかけて体重を増加させる急速増量を行う．試合向け減量を行うアスリートの競技特性には大きく分けて体重階級制，審美系，記録系の競技があり，特に柔道，レスリング，ボクシングなどの体重階級制の競技はルールとして体重の制限がある為に，１週間から数日にかけて厳しい減量を行っている現状にある（久木留ら，2006）．これまでの報告から，競技アスリートが１週間から数日以内で減量前の体重より5%以上を減少させることを短期間の急速減量と定義づけ，急速減量後，数時間から１日にかけて体重を増加させることを急速増量と定義づけた(Oppliger et al., 2006; Oppliger, Steen & Scott, 2003; Steen & Brownell, 1990)

I-II. 急速減量と競技パフォーマンス

競技アスリートの行う減量では競技種目によって目的は異なるものの，いずれも競技力向上を狙って減量を行う．しかし，実際には身体機能や競技パフォーマンスを低下させていることも少なくない．有酸素性作業能への影響は短期間の急速減量と長期間の減量で複

数の論文を比較した際には顕著に現れる．5-36 時間の利尿剤や絶食を用いて行う急速減量

（体重の 1.9-4.9%減少）においては有酸素性作業能の低下が見られるものの(Armstrong,

Costill & Fink, 1985; Webster, Rutt & Weltman, 1990)，12 週間の過体重者が行う減量では有酸

素性作業能の維持や向上が見られる(Kraemer et al., 1999)．しかしながら，体重階級制競技ア

スリートが急速減量後に急速増量を行うことにより，有酸素性作業能や Wingate test などで

示される無酸素性作業能を維持させることができる(Fogelholm et al., 1993)．多くの体重階級

制のアスリートは長期間で減量を行うのではなく，短期間で過度の減量を実施している現

状にある．これら多くの体重階級制アスリートがパフォーマンス低下の危険性があるにも

関わらず急速減量を行うことは特にコンバットスポーツでこの減量・増量方法が有利に働

くと実感しており，急速減量は脱水や飲水制限，絶食を主とした一時的な減量であり，計

量後に時間があれば飲食により体重を増加できるので試合には階級よりも重い体重で出場

し，対戦相手よりも体重面で有利に試合を進めることができることを実感しているからで

はないかと考えられる．

I-III. 体重階級制アスリートの急速減量

　長期間エネルギー収支を負にすることで主に体脂肪を減らす減量では，計量後の大きな

体重増加はなく，この利益を望めない事が急速減量を行う主な要因となっている．米国の

高校生を対象とした調査報告では，シーズン中に減量した選手は約 50%を占め(Kiningham &

Gorenflo, 2001)，その大部分が短期的な急速減量を行っているとの結果であった．さらに，

日本人のレスリング選手を対象にしたアンケート調査によると，男性選手の 90%は 5kg 以

上の減量を実施しているであり，そのうち 72%は 8 日以内の短期間において過度の減量を

実施している（久木留ら, 2006）．

I-IV. 体重階級制アスリートの身体組成

　身体組成の変化は，減量を行う上で栄養摂取や除脂肪量（fat free mass; FFM）の変化がパ

フォーマンスに影響を与えることもあり，アスリートの身体組成を調査した研究は数多く

存在する(Dixon et al., 2006; Dixon et al., 2005; Kordi et al., 2012; Kukidome et al., 2007; Rankin,

Ocel & Craft, 1996)．多くの研究では対象者であるアスリートへの負担を軽減させるため，

二次間接法である生体電気インピーダンス法や皮脂厚法を用いて，身体組成の変化を調査

しており，FFM の多くが減少すると報告している．急速減量には飲水制限が伴うため，体

水分量（total body water; TBW）の急激な減少により脱水状態が引き起こされやすくなる可

能性があり，急激な変化がある場合に正しく身体組成の変化を反映しているかは不明であ

る．したがって，体重階級制アスリートの急速減量中の TBW の変化を考慮した身体組成の

評価が必要である．

I-V. 急速減量とエネルギー代謝

　エネルギー代謝の評価がアスリートのパフォーマンスに必要である一つの理由は，エネ

ルギー出納の結果が体重および身体組成の変化に表れるからである．エネルギー保存の法

則は，エネルギー摂取 (energy intake; EI) とエネルギー消費 (energy expenditure; EE) との関

係が体重や身体組成変化も含めた系において厳密に成り立っている(Elia, Stratton & Stubbs,

2003).水分の変動がなければ,単純に EI が総エネルギー消費量(total energy expenditure; TEE)

を上回れば体重は増加し，逆になれば体重は減少する．しかし，水分の変動があった場合

にはこの限りではないので,TBW の評価はエネルギー出納を考える上で非常に重要である．

同時に，決められた期間でウェイトコントロールを行うには EI と EE の評価が不可欠とな

る．

I-Ⅵ. 研究目的

　本研究の目的は 1) 短期間の急速減量と急速増量を行う競技アスリートの身体組成の変

化を明らかにする， 2) 短期間の急速減量と急速増量を行う競技アスリートのエネルギー

代謝の変化を明らかにすることとした.

第二章

異なる２種類の安定同位体を用いた

短期間の身体組成評価のモデル作成および検討

II-I. 緒言

　急速減量には飲水制限が伴うため，体水分量（total body water; TBW）の急激な減少により脱水状態が引き起こされやすくなる可能性がある(久木留ら, 2006)．脱水状態に陥ると，体液や電解質のバランスが崩れ，筋収縮の異常をはじめ身体に様々な弊害が引き起こされる(渡辺ら, 1984)．従って，安全かつ効率的に減量するためには，減量中及び減量後の TBW に関するデータの蓄積が必要である．

　TBW 測定のゴールデンスタンダードは身体組成推定の為の一次間接法である重水素や重酸素を用いた安定同位体希釈法である．自然界にほとんど存在しない重水素（^2H_2O）または重酸素（$H_2{}^{18}O$）で標識された水を飲水し，その後 3−4 時間に渡り体内の TBW で一定に希釈される．希釈された時間に採尿もしくは採血を行うことにより低侵襲で TBW の推定を行うことが出来る．しかしながら，これらの重水素（^2H_2O）または重酸素（$H_2{}^{18}O$）がターンオーバーするには 2−3 ヶ月程度の期間を有する(Yamada et al., 2009a).

　従って，短期間の TBW の変動を評価する為にはこれら 2 つの異なる安定同位体を用いた TBW の評価が必要となってくる．重水素（^2H_2O）は TBW 以外に 4.1 ％脂肪内に分布し，重酸素（$H_2{}^{18}O$）は 0.7 ％ミネラル内に分布するという特性はあるものの，Racette らが示す，TBW 推定の為の補正を使用すれば同程度の TBW の推定が可能であると考えられる(Racette

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et al., 1994). しかしながら，短期間の TBW 推定の為に異なる 2 種類の安定同位体を用いた

研究はほとんどない.

　そこで本研究の目的を異なる 2 種類の安定同位体を用いた短期間の TBW 推定を検討す

ることとした．加えて，短期間の身体組成の変動を精度よく評価することの出来ると考え

られる 3 成分モデルを検討することとした.

II-Ⅱ. 方法

A. 対象者

若年男性 10 名（年齢; 23.1±1.6 歳, 身長; 171.7±3.6 cm, 体重; 63.6±4.5 kg, BMI; 21.6±1.3 kg/m^2）

を対象とした．研究実施に先立ち，全ての対象者へインフォームドコンセントを実施し，

研究参加同意書への署名を得た．本研究は福岡大学研究倫理委員会の承認（10-12-02）を得

て実施した．

B. 研究デザイン

対象者には 1 回目と 2 回目（First, Second）の身体組成測定の間，体重の変動がないよう

に依頼した．

1 回目と 2 回目の安定同位体投与は 3 日間の間隔をおいた．TBW 測定のための安定同位体

は 1 回目と 2 回目で異なる同位体を使用した．

C. 身体組成測定

形態測定は 12 時間以上の絶食の状態，排尿後に行った．身長は 0.1 cm 単位で測定できる

身長計で測定した．体重は軽装の下着のみでデジタル体重計（Shinko Denshi Vibra Co. Ltd,

Tokyo, Japan）を使用し 0.01 kg まで測定した．水中体重の測定と同時に 100%酸素の再呼吸

法で残気量を推定し，補正した水中体重により身体密度を算出した．体水分量は重水素水

（^2H_2O 99.8 atom%; Taiyo Nippon Sanso, Tokyo, Japan）と重酸素水（$H_2^{18}O$ 10.0 atom%; Taiyo

Nippon Sanso, Tokyo, Japan）の安定同位体を用いて推定した．投与量は体脂肪率（%Fat）を

15%と仮定し，除脂肪量に 0.732 の定数を乗し，体水分量を推定した値を元に ^2H_2O であれ

ば 0.12 g/kg，$H_2^{18}O$ であれば 2.5 g/kg の投与を行った．$^2H_2$ は白金を触媒として 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，$^2H_2$, ^{18}O の安定同位体比を質量比分析計

（SerCon Isotope Ratio Mass Spectrometers CF 20-20; Sercon Ltd, Crewe, UK）により分析した

(Yamada et al., 2009b). 尿中の安定同位体比から，標準化した安定同位体濃度は，［a (ds －

db)］／［WA (da － dt)］で求められる．ただし，W は同位体比分析の際に 安定同位体を

希釈するのに用いた飲料水の量(g)，A は投与した安定同位体の量(g)，da は希釈した安定同

位体における同位体比，dt は安定同位体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安定同位体の量(g)，ds は尿中の同位体比，db は投与前の尿の

同位体比である．求められた体水分量は ^2H であれば 1.041 で除し，^{18}O であれば 1.007 で除

し補正を行った(Racette et al., 1994).

　対象者は身体組成の測定日早朝に来研してすぐ採尿を行い，その後希釈された安定同位

体を飲水した．安定同位体の飲水後の 2,3,4 時間後に採尿を行い，分析には安定同位体が体

の全体に平衡したと考えられる 3,4 時間尿を使用し,1 サンプル 3 回の分析を行った．体水分

量は 3 時間と 4 時間を平均した数値を採用した．

%Fat は Siri の 3 成分モデルの式を用いて算出した(Siri, 1993)．算出した%Fat より体脂

肪量を算出し，体重より体脂肪量と体水分量を引いた値を除脂肪固形分量として算出した．

D．統計処理

結果は平均値 ± 標準偏差で示した．全ての統計処理は Microsoft Excel 2010 を用いて行っ

た．2 群間の平均値の差の検定には対応のある t 検定を用いた．尚，有意水準は $p < 0.05$ に

設定した．

II-Ⅲ. 結果

3 成分モデルで評価された身体組成は，各項目において，有意な変化を認めなかった（体重; －0.2 ± 0.5 kg, p = 0.17, %Fat; －0.1 ± 0.5 %, p = 0.49, 体脂肪量; －0.1 ± 0.4 kg, p = 0.36, 体水分量; －0.1 ± 0.4 kg, p = 0.56, 除脂肪固形分量; 0.0 ± 0.4 kg, p = 0.71）．変動係数は，いずれの項目において 3%未満であった(Table 1).

II-Ⅳ．考察

　本研究の目的は異なる２種類の安定同位体（重水素水と重酸素水）を用いた短期間の TBW

推定法および３成分モデルの検討であった．

　体重及び身体組成の有意な変化を認めなかった．各測定の変動係数は，0.5-2.2%を示し，

最も大きい変動係数は体脂肪量と%fat であった．身体組成の精度検定を行った研究による

と，再テスト法による変動係数 2.3 ± 1.9%と報告されている(McCrory et al., 1995). 本実験で

の変動係数は，先行研究の値の範囲内であり，信頼性のある値として考えられる．

　体重階級制アスリートの体重変動は減量前体重の 5 ％以上減少し，脱水は 2%以上起こる

ことが予想される（森本ら, 2007）．1 週間の短期間で異なった安定同位体を用いた TBW の

測定で身体組成を評価した場合も結果が示すように，３成分（体脂肪量，体水分量，除脂

肪固形分量）を精度よく評価することが可能であると考えられる．

II-V. 結論

　　異なる２種類の安定同位体を用いることで短期間の TBW 推定を行うことができる可

能性が示唆された。加えて，短期間の身体組成の変動を 3 成分モデルを用いることで評価

することが出来る可能性が示された．

第三章

急速減量と急速増量が身体組成と

エネルギー代謝に与える影響の検討

Ⅲ-I. 緒言

　柔道，レスリング，ボクシングなどの体重階級制の競技はルールとして体重の制限がある為に，厳しい減量を行っている．体重階級制のアスリートは通常の体重よりも低い階級で試合に臨む選手が多く，1週間程度の短期間で体重を減少させる急速減量を行い，その後数時間から1日で体重を増加させる急速増量を行う傾向にある(Oppliger et al., 2006; Oppliger et al., 2003; Steen & Brownell, 1990).

　急速減量には飲水制限が伴うため，体水分量（total body water; TBW）の急激な減少により脱水状態が引き起こされやすくなる可能性がある(久木留ら, 2006). 脱水状態に陥ると，体液や電解質のバランスが崩れ，筋収縮の異常をはじめ身体に様々な弊害が引き起こされる(中野ら, 1998). 従って，安全かつ効率的に減量するためには，減量中及び減量後の TBW に関するデータの蓄積が必要である．TBW 測定のゴールデンスタンダードは一次間接法である重水素や重酸素を用いた安定同位体希釈法である.例えば,肥満者の減量における TBW の変化に関してはいくつかの研究があるものの(De Luis et al., 2009; Siervo et al., 2010)，減量前の体脂肪率が正常であるアスリートの急速減量下の TBW の変化を安定同位体希釈法で調べた研究は我々の知る限りみられない．いくつかの先行研究では，二次間接法である皮下脂肪厚計測法や生体電気インピーダンス（bioelectrical impedance analysis; BIA）法といっ

た方法を用いて急速減量中の身体組成の変化を調査しているが(Rankin et al., 1996)，これら

の二次間接法では多くの仮定が必要で，急速減量など急激な変化がある場合に正しく体組

成の変化を反映しているかは不明である．したがって，体重階級制アスリートの急速減量

中の TBW の変化を安定同位体希釈法を用いて明らかにすることが必要である．

　各種スポーツ種目ではそれぞれ適正な体脂肪率が存在するため，体重減少に占める体脂

肪量（fat mass; FM）と除脂肪量（fat free mass; FFM）の割合（FM:FFM 比）を明らかにする

ことは，最高の競技パフォーマンスを維持しながら目標とする体重減少を達成する上で重

要である．身体組成を推定する一次間接法としては，水中体重法（under water weight; UWW）

法，安定同位体希釈法，二重エネルギーX 線吸収（dual-energy x-ray energy absorptiometry; DXA）

法，核磁気共鳴画像法（magnetic resonance imaging, : MRI）などが存在するが，すべての方

法で仮定が存在し，急速減量のように FFM に占める TBW の割合が変化しうる条件では，

どの単一の測定法でも正しく体脂肪率を評価することができない．したがって，UWW 法と

安定同位体希釈法を組み合わせる 3 成分モデルや，それにさらに DXA 法を組み合わせる 4

成分モデルなどが体脂肪率推定のゴールデンスタンダード法として用いられる．しかし，

体重階級制のアスリートの急速減量およびその後の急速増量における FM:FFM 比の変化を

多成分モデルで評価した研究はない．このようなデータを提示することは，体重階級制の

スポーツ選手への適切な栄養や運動・減量指導を行う上で重要と考えられる．4 成分モデル

と 3 成分モデルとの差異は，DXA で骨塩量を測定するかどうかであるが，急速減量中の骨

塩量の変化量は，1-100g 未満であり，このような微細な変化は FM や FFM を推定する上で

大きな影響を与えないことから(Karila et al., 2008; Santos et al., 2010)，急速減量中の FM や

FFM の変化を明らかにするうえでは，3 成分モデルでも妥当性が高い．そこで，本研究で

は， UWW 法と安定同位体希釈法による 3 成分モデルにより，急速減量およびその後の増

量期の FM と FFM の変化を明らかにすることを第一の目的とした．

　加えて，FFM に含まれる骨格筋と基礎代謝（basal metabolic rate; BMR），睡眠時代謝

（sleeping metabolic rate; SMR）などの安静時のエネルギー消費量は高い相関関係にあり

(Ganpule et al., 2007)，FFM の減少がこれらのエネルギー消費量に影響を及ぼす可能性があ

る．レスリング選手を対象とした 1 週間で 2.9 kgの急速減量を行った先行研究での報告は

FFM 1kg 当たりの BMR および SMR が有意に減少するとしている(Kukidome et al., 2007). し

かしながら，この先行研究では FFM を BIA 法で測定しており，上述のように妥当性に欠け

るため，再検討が必要である．また，実際には急速減量後に急速増量して競技に臨むが，

急速減量後に回復した際のエネルギー消費量と身体組成について検討した研究は我々の知

る限り見当たらない．そこで本研究では短期間での急速減量と急速増量が BMR，SMR，に

及ぼす影響を明らかにすることを第二の目的とした.

III-II. 方法

A. 対象者

体重階級制競技経験(ボクシング，柔道)のある若年男性 10 名(年齢 20 ± 1.4 歳)を本研究の

対象とし，福岡大学倫理委員会の承認（10-11-1）を受けて行った．対象者に本研究の目的

および測定内容を説明した後，各対象者から研究参加への承諾を面談と書面により得た．

B. 研究デザイン

本研究は，急速減量前の通常体重時(通常期, Baseline; BL)，急速減量後の減量時体重(減量

期, Wegiht loss; WL)，急速増量の増量時体重(増量期, Weght regain; WR)で 3 回の測定を順に

行った．

対象者には急速減量前の通常期の体重より 1 週間で体重の 5%を目標に減量を行い，その

後 1 日で体重を減量前の体重に戻してもらうように指示した．脱水法 (サウナ, 飲水制限)，

食事制限，活動量の増大による減量が考えられるが，減量方法は対象者に一任した．詳細

の実験方法は Table 2 に示した．

C. 身体組成測定

身体組成の測定はメタボリックチャンバーに入室する日の早朝に 12 時間以上の絶食の状

態，排尿排便後に行った．身長は 0.1cm 単位で測定できる身長計で測定された．体重は軽

装でデジタル体重計(Shinko Denshi Vibra Co. Ltd, Tokyo, Japan)を使用し 0.01kg まで測定され

た．水中体重の測定と同時に 100%酸素で再呼吸を行い，残気量を推定し，補正した水中体

重により身体密度を測定した．TBW は重水素(^{2}H$_2$O 99.8 atom%; Taiyo Nippon Sanso, Tokyo,

Japan)と重酸素(H$_2$^{18}O 10.0 atom%; Taiyo Nippon Sanso, Tokyo, Japan)の安定同位体を用いて

推定した．投与量は体脂肪率を 15%と仮定し，除脂肪量に 0.732 の定数をかけて TBW を推

定した値を元に，^{2}H$_2$O であれば 0.12g/kg，H$_2$^{18}O であれば 2.5g/kg の投与を行った．^{2}H$_2$ は白

金を触媒として 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$_2$,^{18}O の安

定同位体比を質量比分析計（SerCon Isotope Ratio Mass Spectrometers CF 20-20, Sercon Ltd,

Crewe, UK）により分析した(Yamada et al., 2009b) 尿中の安定同位体比から，標準化した安

定同位体濃度は，［a（ds－db）］/［WA（da－dt）］ で求められる．ただし，W は同位体比

分析の際に 安定同位体を希釈するのに用いた飲料水の量（g），A は投与した安定同位体の

量（g），da は希釈した安定同位体における同位体比，dt は安定同位体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 安定同位体の量（g），ds は尿

中の同位体比，db は投与前の尿の同位体比である．求められた TBW は ^{2}H であれば 1.041

で除し，^{18}O であれば 1.007 で除し補正を行った(Racette et al., 1994).

　　対象者は身体組成の測定日早朝に来研してすぐ採尿を行い，その後希釈された安定同位

体を飲水した．安定同位体の飲水後の 2,3,4 時間後に採尿を行い，分析には安定同位体が体

の全体に平衡したと考えられる 3,4 時間尿を使用し,1 サンプル 3 回の分析を行った．TBW

は 3 時間と 4 時間を平均化したものを採用した．

　　体脂肪率(%Fat)は Siri の 3 成分モデルの式を用いて算出した(Siri, 1993). 算出された%Fat

より体脂肪量を算出し,体重より FM と TBW を引いた値を除脂肪固形分量(free fat dry solid；

FFDS)，TBW と FFDS を足した値を FFM として算出した．

D.身体活動レベルと食事摂取量

　　身体活動レベル(physical activity level；PAL)の評価は, 3 軸加速度計(Panasonic Electric

Works Co., Ltd, Osaka,Japan)を用いた(Yamada et al., 2009b). 対象者は, 通常期の測定の 1 週

間前より測定を開始し，回復期の測定終了まで装着した．解析には各期間の活動量の平均

値を用いた．

　　対象者は配布された自己記入式の食事調査票に通常期（減量期間の 1 週間前）から減量

期（減量期間の 1 週間）と増量期（体重回復の 1 日）の食事内容及び間食内容を記録した．

記入上の注意事項に関しては対象者に事前に十分説明を行い，注意事項の書かれた用紙を渡した．食事の自己記入と同時に対象者はカメラ機能付きの携帯電話もしくはデジタルカメラにより写真撮影を行い，すべての測定が終わった際に，食事調査票と写真を回収し管理栄養士が聞き取りを実施した．記録された内容は栄養価計算ソフト（エクセル栄養君 Ver. 4.5）を用いて，エネルギー及び各栄養素摂取量を算出した．

E. メタボリックチャンバー

　　メタボリックチャンバーはヒトのエネルギー代謝を測定する間接熱量法の一種であり，マスク法やフード法などの活動の制限を受けるような方法とは対照的に，長時間測定ができる装置である．構造的に密閉性が確保されている部屋全体の空気を連続して測定している．本研究室は Pull タイプのカロリーメータであり，管から取り入れられた外気は一定状態(25℃, 湿度 50%)に調整された後に室内に供給され，部屋内で十分に混合された後，排出管から一定量(80L / min)吸引され，排出口に取り付けられたガス分析センサーと流量計(CMS0200 Gas Mass Flow Meter，山武社，東京，日本)にて測定される．なお，室内への空気の流入量と排出量は同じになるようにコントロールされている．排気速度は質量流量制御装置で制御され，室内より排出される空気中のガス濃度はオンラインのプロセス用質量分

析計(VG PRIMA δB, Thermo Fisher Scientific, Cheshire, UK)で測定された．

　測定により得られたガス濃度を元に，Henning の式に基づき毎分の酸素摂取量と二酸化炭素排出量を計算し(Henning, Löfgren & Sjöström, 1996)，Weir の式に基づいて毎分の エネルギー消費量（kcal / min）を算出した(Weir, 1949)．また，本 メタボリックチャンバーは定期的にアルコール燃焼試験（3 時間）を行い，測定値／理論値が酸素 99.97％，二酸化炭素 100.03％程度であるため，高い精度での長時間にわたるエネルギー消費量の連続測定が可能である．

F. メタボリックチャンバープロトコル

　対象者は測定の前日から運動後余剰熱産生のエネルギー消費量への影響を避けるために激しい運動を行わないように指示された．カロリーメータ内では座位安静とし，主な時間は読書，テレビ観賞，書き物をして過ごした．対象者は起きた状態を維持し，睡眠時間以外は寝ないように監視された．測定日の夕方(17:00)から対象者はメタボリックチャンバーに入室した．17:40 に対象者は各期間に合わせ，各自で持ってきた自由食を摂取し始め，18:00 に食べ終わるようにした．23:00 に就寝し 6:00 に起床した．6:00 に起床してからは BMR を測定するために仰臥位で 6:30 まで過ごしてもらった．その間に対象者が寝ないように監視

した．その後，6:40 に朝食を食べ始め 7:00 丁度に朝食を食べ終え，その後 3 時間座位安静

を保ち，10:30 にメタボリックチャンバーから退出した．(Table 2)

G. 統計処理

　すべての統計処理は統計パッケージ(SPSS 18.0 for Windows)を用いて行った．すべての

数値は平均値 ± 標準偏差で示した．各測定項目の通常期, 減量期, 増量期で一元配置分散

分析(ANOVA)を行い主効果が認められたものには，Tukey b 法を用いて，各群間の差の検定

を行った．統計学的有意水準は 5%未満とした． 体重と FFM の減少が BMR と SMR への与

える影響を取り除く為に，繰り返しのある共分散分析(ANCOVA)が体重、FFM を共変量と

し，通常期，減量期，増量期で BMR または SMR を比較するために行われた．

Ⅲ-Ⅲ. 結果

A. 身体組成

Table3 で示すように，体重は通常期から減量期にかけて有意に減少し (-6.0 ± 0.9%)，その後の増量期に増加した．しかしながら，増量期の体重は通常期の体重まで増加しなかった．TBW は通常期から減量期にかけて有意に減少し (-5.1 ± 1.8%)，その後の増量期に増加した．FFDS は通常期に対し減量期に有意に減少したが (-3.9 ± 1.5%)，その後の増量期に増加した．FM は通常期から減量期にかけて有意に減少し (-15.5 ± 12.1%)，増量期で増加したが， 通常期の FM に比べ有意に低値であった(-10.0 ± 6.5%)．急速減量の体重減少に寄与した FM と FFM の割合として，FM が 33.8 ± 13.6%，FFM が 66.2 ± 13.6%であった．急速増量に寄与していた FM と FFM の割合は FM が 9.2 ± 32.3%，FFM が 90.8 ± 32.3%であった(Table3).

B. エネルギー消費量

1 日当たりに換算した BMR は通常期に対して減量期で減少する傾向にあったが(P=0.09)，増量期では通常期と有意な変化は見られなかった．また，体重と FFM で補正した BMR はすべての期間において有意な変化は見られなかった(Table4).

SMR は通常期と比較したときに減量期で有意な減少を示したが,体重増加に伴い通常期

まで増加した．体重と FFM で補正した SMR は通常期に比べ減量期では有意な減少を示したが，その後の急速増量で増加した(Table4)．

C. 身体活動レベルとエネルギー摂取量

通常期から増量期にかけてのエネルギー摂取量及び PAL を Table 5 に示した．エネルギー摂取量は通常期に比べ減量期で有意に減少し，増量期で有意に増加した．たんぱく質，脂質，糖質の 3 大栄養素はすべての項目で通常期に比べ減量期で有意に減少し，増量期で有意に増加した．PAL に関して，通常期と減量期で有意な変化は見られなかったが，通常期に対して増量期で 1.40 ± 0.17 と有意に減少した．

Ⅲ-Ⅳ．考察

　本研究の主な知見は体重階級制のアスリートが行う典型的な急速減量とその後 1 日の増量法による体重の増減には主に TBW と FM が寄与していることである．本研究では体重階級制アスリートが行う急速減量と急速増量の身体組成の変化，特に FM:FFM の減少の比率と TBW の変化に着目し検討を行った．その結果，減量前と比べて急速減量後に体重が 6%減少し，減量後の増量で体重が 4.6%増加した．体重減少の 33.8%が FM で 66.2%が FFM であった．体重増加の 9.2%が FM で 90.8%が FFM であった(Table 3)．3 成分モデルで示すと急速減量による体重の変化の 50.6%が TBW であり，33.8%が FM，15.6%が FFDS の減少であった．減量後の増量では体重増量の 67.5%が TBW であり，9.2%が FM，23.3%が FFDS の増量であった．レスリング選手の身体組成の評価に MRI を用いた先行研究では， 1 週間での急速減量により 5.9%の大腿部の骨格筋横断面積が減少したが，計量後，増量を行い試合に臨んだ後の測定では 1 週間前に測定した骨格筋横断面積と同等まで回復している(Kukidome et al., 2008)．この先行研究では，計量後と試合前までの飲食と飲水の増加により骨格筋横断面積が増加したとしているが，重要であると考えられる TBW の測定を行っていない．そのため，本研究の結果は先行研究では測定されていない TBW の結果を補完するものであると考えられる．

本研究での通常期に対する減量期の FFDS の 0.7kg が減少していた．FFDS の組成はグリ

コーゲン，ミネラル，タンパクで構成される．先述したように急速減量中のミネラルの変

化は微量であり，100g 精度で骨塩量を測定した先行研究では変化が見られない(Karila et al.,

2008)．この成分中のグリコーゲンは水の分子とのモル比率が約 1:3 で水和し，構造上水を多

く含む(King et al., 2008)．また，グリコーゲンは骨格筋と肝臓に含まれることはよく知られ

ており，体水分の変化とグリコーゲンの変化に関連がある研究も存在する(Fenn & Haege,

1940; Olsson & Saltin, 1970; Puckett & Wiley, 1932)．全身のグリコーゲン量は体重が 80kg で

体脂肪率が 15%であれば，血中(20g)，肝臓(100g),骨格筋(400g)の器官に 520g 程度存在する

(Jeukendrup, 2003)．骨格筋グリコーゲンが水分子と約 1:3 で水和しているのならば，TBW が

約 1.2kg 貯蔵されていることになる．Tarnopolsky らの研究ではレスラーの急速減量後に筋

グリコーゲンが減量前より 54%有意に減少したにも関わらず，減量後 17 時間の増量で減量

前の値と差を認めない事を報告している(Tarnopolsky et al., 1996) これらの研究を考慮する

と 0.648kg の骨格筋グリコーゲン量が減少していることになり我々の体重 74.4kg である

FFDS の減少量 0.7kg と近似する．従って，本研究において，TBW が減量後に大幅に減少し，

増量後に増加したことを考えると，TBW の変化にはグリコーゲンの変化が寄与しており，

FFDS の減少はグリコーゲンの減少ではないかと推察される．

本研究において，FM が急速減量期に平均 1.5kg 減少した．BMR と加速度計から評価した

PAL で評価した推定エネルギー消費量は，減量期で平均 3490 ± 560 kcal/day であり，食事調

査票から評価した推定エネルギー摂取量は，減量期で平均 1008 ± 354 kcal/day であった．こ

れらの結果から FM の減少を推定すると 1.89 kg となり，真の減少量に近似する．すなわち，

FM の減少はエネルギー出納バランスを負にした結果であり，1 週間での急速減量の有効性

を示している．急速減量では減量期に身体活動の増大による減量が考えられるが，本研究

では通常期と減量期で PAL に変化は見られなかった．この結果から，本研究の減量は食事

制限による FM の減少が大きかったと考えられる．

　本研究で BMR は各期間で有意な変化を示さなかった．長期間の減量であれば，食事制限

をおこなうと FFM の低下に伴い BMR が低下することが報告されている(Melby, Schmidt &

Corrigan, 1990)．体重階級制アスリートにおいても同様に安静時代謝が減量に伴い低下して

いる(Forbes, 2000; Kukidome et al., 2007)．これらの研究では，FFM の減少が安静時代謝の低

下に寄与していると推察しているが，身体組成の評価を二次間接法である BIA を用いてい

る為に，正確な変化を追えていない可能性がある．肥満者の減量であれば，FF:FFM が一定

の割合で減少し，FFM の減少が BMR に影響を与えている可能性がある(Forbes, 2000)．本研

究のアスリートは短期間で FM:FFM が約 1:2 とより多くの FFM が減少しているが，FFM の

減少のうちの大部分が TBW であったことから，本研究では先行研究と異なり，BMR が急速減量(P=0.09)や急速増量により影響を与えなかった可能性が考えられる．また，本研究では TBW 測定のゴールデンスタンダードである安定同位体希釈法を用いたが， TBW の増減に BMR の変化が伴わなかったことから，TBW の増減は BMR に影響を与えないかもしれない．しかしながら，TBW が BMR に与える因果関係については明らかではなく，我々が知る限り見当たらない．今後は TBW を細胞内液と細胞外液に分けた詳細な検討が必要である．

BMR の結果とは異なり，SMR は通常期と比較し減量期に有意に減少していた (-235kcal / day)．SMR は 48 時間の絶食で SMR が減少するという研究や(Weyer et al., 2001), 食事制限や減量に伴い食事誘発性体熱産生 (diet-induced thermogenesis; DIT) が低下する(Platte et al., 1996)という研究がある．本研究において，通常期や増量期と比較し，減量期の食事摂取は平均 984kcal/day であったこともあり，SMR が減少した要因として，厳しい食事制限の影響が考えられる．特に，DIT は食後 6 時間もしくはそれ以上続くという研究も報告されていることから(Westerterp, 2004)，食後 5 時間からの解析では大きく SMR の結果に影響しているが，食後 12 時間からの BMR の解析結果への影響は少ない可能性が考えられる．これらの要因が BMR と SMR を異なる結果に導いた一つの要因である可能性がある．本研究のメタボリックチャンバーで測定した食後 3 時間の DIT は通常期と比較して減量期で約 49%減少

しており，この DIT の影響が通常期および増量期の睡眠時間にまで残存していた可能性が否定できない(Table 4)．また，BMR と SMR のエネルギー消費量の違いを検討した研究において，8 時間の SMR と BMR の比較では約 1%の誤差であり，SMR の最小の 3 時間と基礎代謝を比較しても誤差は 6%程度であり近似した結果を示している(Ganpule et al., 2007)．Kumahara らの日本人を対象に SMR を検討した研究において男性での SMR／BMR の比率は 93%であった(Kumahara et al., 2004)．本研究における SMR／BMR の比率は通常期に 91.2%であり，増量期に 93.6%であるのに対して，減量期では 86.0%と明らかに低い値を示していることからも減量期の DIT 低下の影響が示唆される．

　本研究の限界として，減量経験や減量回数，ウエイトサイクリング等がエネルギー消費量に影響する可能性も考えられるが，本研究では 1 回の急速減量と急速増量での変化をより正確に測定するため，ウエイトサイクリング等の影響を考慮しなかった．この問題に関しては，減量経験や減量回数，減量方法のアンケートで検討する必要があると考えられる．また，急速減量後の回復期間は競技種目によって数時間から 1 日まで異なるが，より正確な身体組成およびエネルギー消費量の測定を行うために，1 日に設定した．対象者の身体組成に関しては，急速減量と急速増量の増減には TBW の影響が大きいことが明らかになったが，実際にはグリコーゲン量や骨を形成するミネラルおよび骨格筋を形成するタンパク

の測定を行っていない．従って，通常期から減量期にかけて減少し，減量期から増量期に

かけて増量した FFDS がこれらの成分中の何であるかは明らかではない．

Ⅲ-Ⅴ．結論

　　本研究では急速減量と急速増量による体重の増減には体水分の変化が大きく影響して

いることが明らかになった．基礎代謝は短期間での急速減量と急速増量において，大きく

変動しない可能性が示唆された．

第四章

結論

体重階級制アスリートにおける３成分モデルで示される急速減量とその後の１日の急速

増量の主な身体組成成分は体水分と体脂肪であることが明らかとなった．

　基礎代謝は短期間での急速減量と急速増量において，大きく変動しない可能性が示唆され

た．また，体重階級制のアスリートが行う 1 週間程度の短期間の減量と１日での増量法は

除脂肪固形分を維持しつつ体脂肪量を減らす為の有効な方法であると考えられた．

第五章

本研究の限界

これまでの研究では短期間の急速減量と急速増量に伴う身体組成の変化を明らかにした

が，方法や対象者は限定的であり，これらの結果はいずれも一過性の急速減量によるもの

に限られる．　また，減量中には運動量の増加が考えられるため，運動中のエネルギー消費

量や基質酸化の変動をとらえる取り組みが必要となってくる．

　今後の研究では，急速減量の多様性を広げより一般化しやすい減量様式を検討するため，

肥満者からトップアスリートを対象に継続して検討する必要がある．さらに、体重階級制ア

スリートにおいてはウェイトサイクリングを考慮するため，競技特性や急速減量回数のデ

ータ蓄積が必要となってくると考えている．

第六章

図表

Table1. Changes in body composition and coefficient of variation

	First	Second	C.V (%)
Body weight (kg)	63.6 ± 4.5	63.4 ± 4.1	0.5 ± 0.3
Percent of fat (%)	14.5 ± 3.0	14.4 ± 3.0	2.2 ± 1.7
Fat mass (kg)	9.3 ± 2.5	9.2 ± 2.5	2.2 ± 2.1
Fat free dry solid (kg)	15.5 ± 0.8	15.5 ± 0.7	1.3 ± 0.9
Total body water (kg)	38.7 ± 2.1	38.7 ± 2.0	0.6 ± 0.3

There are no significant between First and Second on all of these.

Table 2 . Timetable for the modeling day in the metabolic chamber (MC)

Time	Action
0630	Arrive for body composition measurement
0640	Urine sample collection at baseline
0650	Administer stable isotope
0700	Underwater weighing
0730	Arrive at home
0850	Urine sample (2 hour)
0950	Urine sample (3 hour)
1050	Urine sample (4 hour)
1700	Entry into MC
1740	Dinner (self-selected diet)
1800	Sit quietly
2300	Go to sleep → SMR (2300-0600)
0600	Wake up → BMR (0600-0630)
0630	Get up
0640	Breakfast (prescribed diet)
0700	Sit quietly
1030	Exit from HC

Table 3. Changes in body composition in the 3-component model

	Baseline	Weight loss	Weight regain
Age (yrs)	20.0 ± 1.4	—	—
Height (cm)	174.6 ± 6.8	—	—
Body weight (kg)	74.4 ± 9.0	70.0 ± 8.8*	73.1 ± 8.6*[#]
		(-4.4 ± 0.6)	(-1.3 ± 0.8)
% Fat	17.0 ± 7.4	15.8 ± 7.6*	15.9 ± 8.2*
		(-1.2 ± 1.0)	(-1.1 ± 1.2)
FM (kg)	13.1 ± 7.3	11.7 ± 7.5*	12.1 ± 7.3*[#]
		(-1.5 ± 0.6)	(-1.1 ± 0.8)
FFM (kg)	61.2 ± 4.2	58.3 ± 3.8*	61.1 ± 3.7[#]
		(-2.9 ± 0.8)	(-0.2 ± 0.9)
TBW (kg)	43.8 ± 3.1	41.5 ± 2.9*	43.6 ± 2.6[#]
		(-2.3 ± 0.9)	(-0.2 ± 1.0)
FFDS (kg)	17.5 ± 1.2	16.8 ± 1.1*	17.5 ± 1.2[#]
		(-0.7 ± 0.3)	(0.0 ± 0.3)

Values are mean ± standard deviation ($n = 10$). The case arc is the amount of change vs. baseline. *$p < 0.05$ vs. baseline; [#]$p < 0.05$ vs. weight loss. FM, fat mass; TBW, total body water; FFDS, fat-free dry solids. FFM shows the sum of TBW and FFDS.

Table 4. Changes in energy expenditure

	Baseline	Weight loss	Weight regain
BMR (kcal/day)	1998 ± 242	1846 ± 261 †	1852 ± 243
BMR adjusted by weight (kcal/day)[a]	1969 ± 220	1885 ± 213	1843 ± 190
BMR adjusted by FFM (kcal/day)[b]	1945 ± 132	1943 ± 209	1809 ± 79
SMR (kcal/day)	1822 ± 191	1587 ± 150 *	1734 ± 222[#]
SMR adjusted by weight (kcal/day)[a]	1798 ± 169	1620 ± 88 *	1725 ± 179
SMR adjusted by FFM (kcal/day)[b]	1786 ± 100	1654 ±116 ††	1704 ± 169
DIT (kcal/h)	23 ± 8	11 ± 7 *	19 ± 5[#]

Values are mean ±standard deviation ($n = 10$).
*$p < 0.05$ vs. baseline; †$p = 0.09$ vs. baseline; ††$p = 0.07$ vs. baseline; [#]$p < 0.05$ vs. weight loss. BMR, basal metabolic rate; SMR, sleeping metabolic rate; FFM, fat-free mass (including total body water); DIT, diet- induced thermogenesis.
DIT was obtained from the difference in the postprandial resting metabolic rate and BMR for three hours at one meal.
[a] Estimated marginal means presented in this row is calculated by ANCOVA with weight as covariates set at 60.2 kg.
[b] Estimated marginal means presented in this row is calculated by ANCOVA with FFM as covariates set

at 72.5 kg.

Table 5.   Energy intake and physical activity level (PAL)

		Baseline	Weight loss	Weight regain
Energy intake	(kcal/day)	2458 ± 578	1008 ± 354*	3066 ± 506*[#]
Protein	(g/day)	86 ± 22	31 ± 10*	101 ± 19[#]
	(g/kg/day)	1.17 ± 0.33	0.45 ± 0.15*	1.39 ± 0.24[#]
	(%)[a]	14 ± 1	12 ± 3	13 ± 2
Fat	(g/day)	79 ± 24	28 ± 13*	113 ± 24*[#]
	(%)[a]	29 ± 5	23 ± 9	32 ± 6[#]
Carbohydrate	(g/day)	334 ± 88	148 ± 57*	396 ± 81*[#]
	(%)[a]	55 ± 5	58 ± 15	53 ± 8[†]
PAL		1.88 ± 0.13	1.93 ± 0.14	1.40 ± 0.17*[#]

Values are mean ± standard deviation ($n = 10$) *$p < 0.05$ vs. baseline, [#]$p < 0.05$ vs. weight loss, [†]$p = 0.05$ vs. weight loss. PAL, physical activity level.
[a] Percentage of the total energy intake.
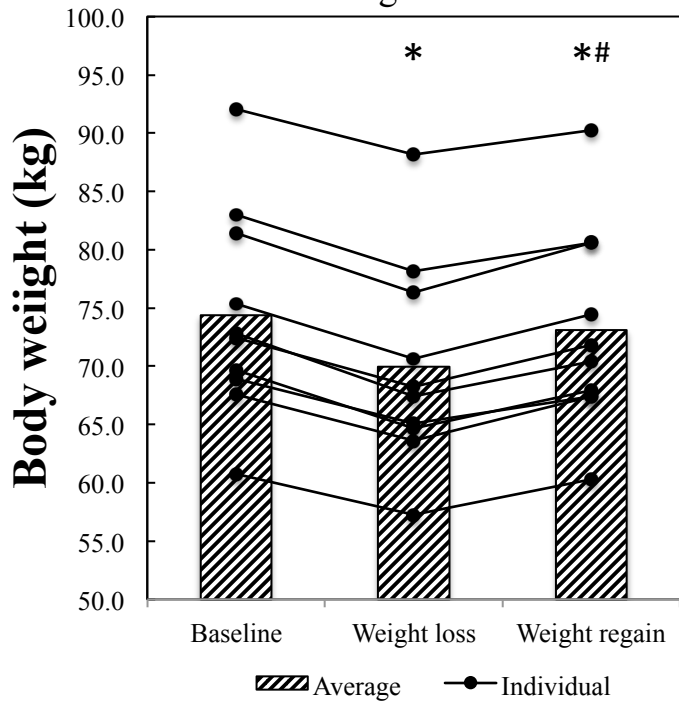
Figure 1 Change in body weight and composition
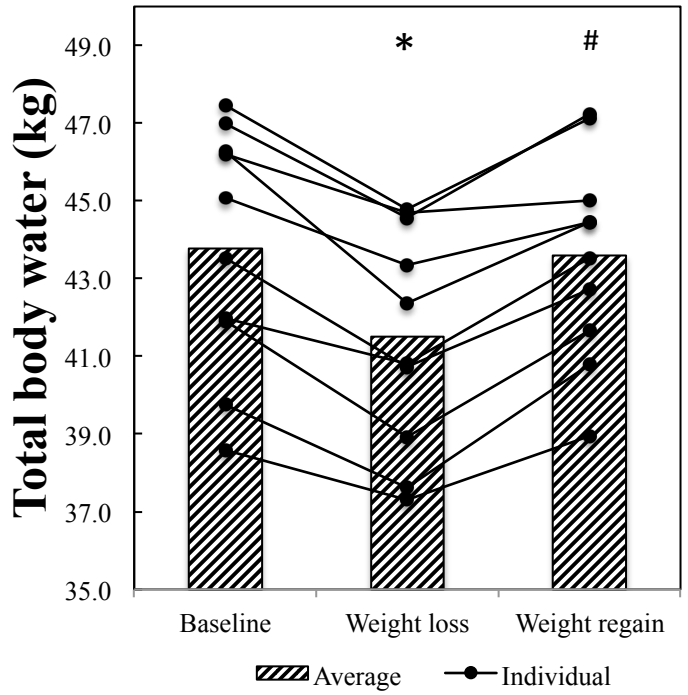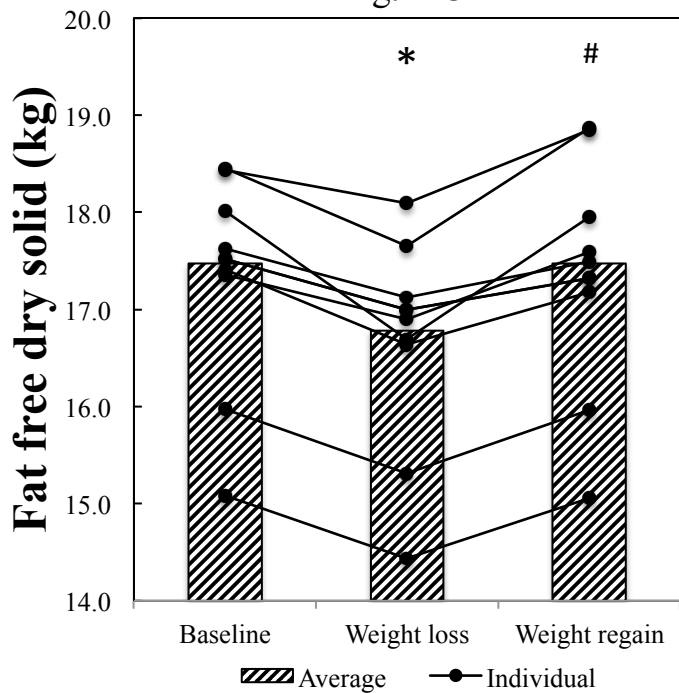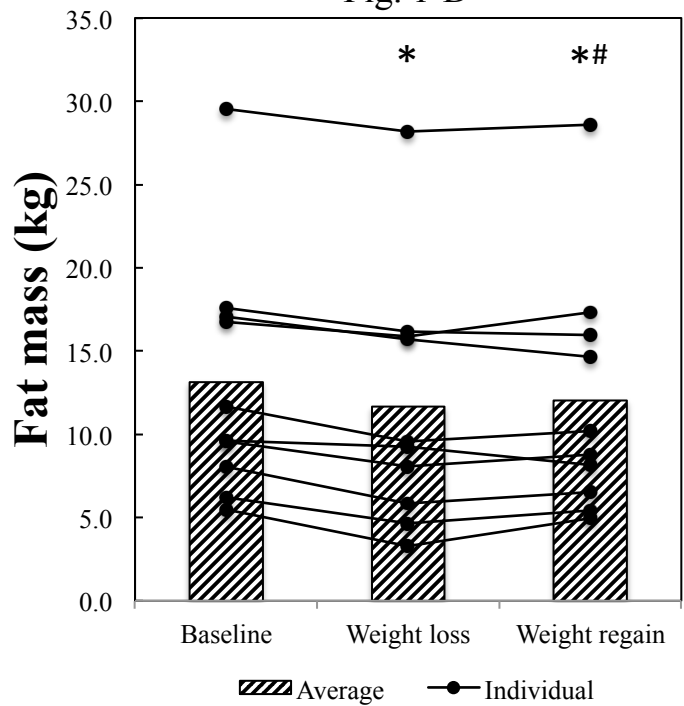

Fig. 1-A

Fig. 1-B

Fig. 1-C

Fig. 1-D

($*p < 0.05$ vs. baseline; $^{\#}p < 0.05$ vs. weight loss.)

Figure 2 Change in Basal metabolic rate
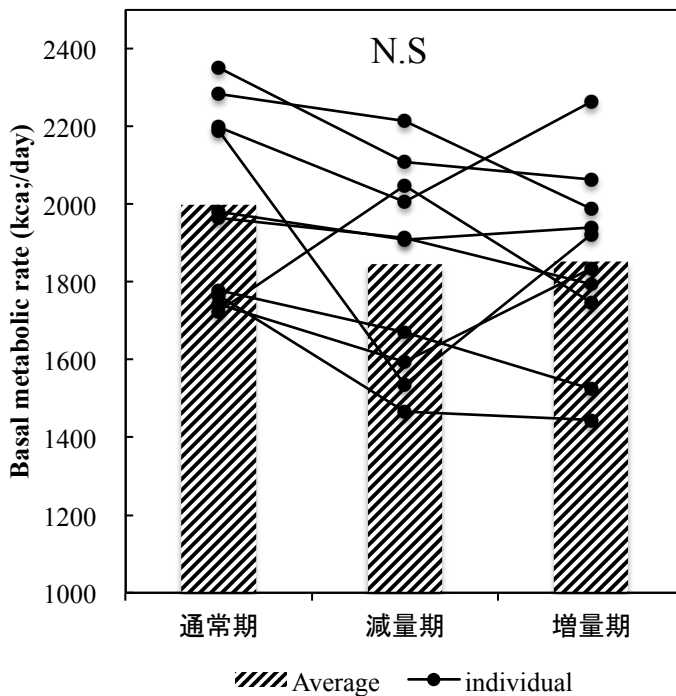
Fig. 2-A

Fig. 2-B

Fig. 2-C

*$p < 0.05$ vs. baseline;

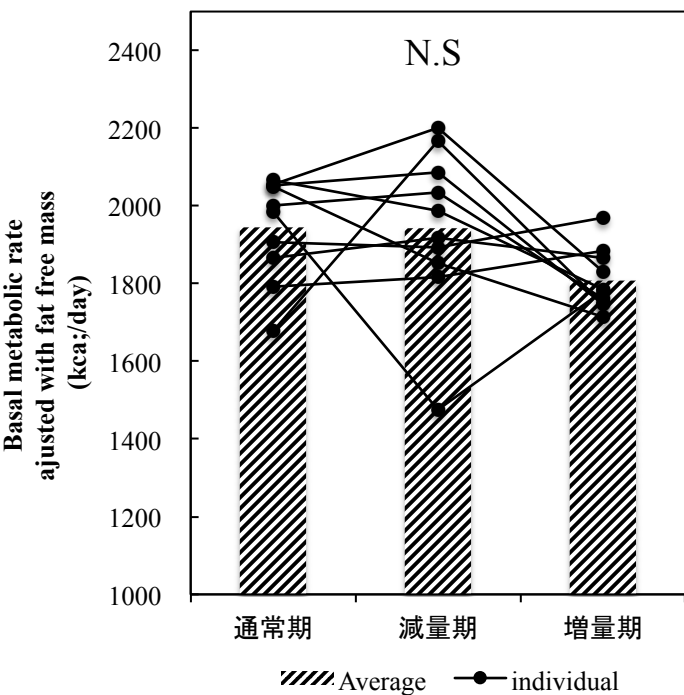[a] Estimated marginal means presented in this row is calculated by ANCOVA with weight as covariates set at 60.2 kg.

[b] Estimated marginal means presented in this row is calculated by ANCOVA with FFM as covariates set at 72.5 kg.

Figure 3 Change in sleeping metabolic rate

Fig. 3-A

Fig. 3-B

Fig. 3-C

*$p < 0.05$ vs. baseline;

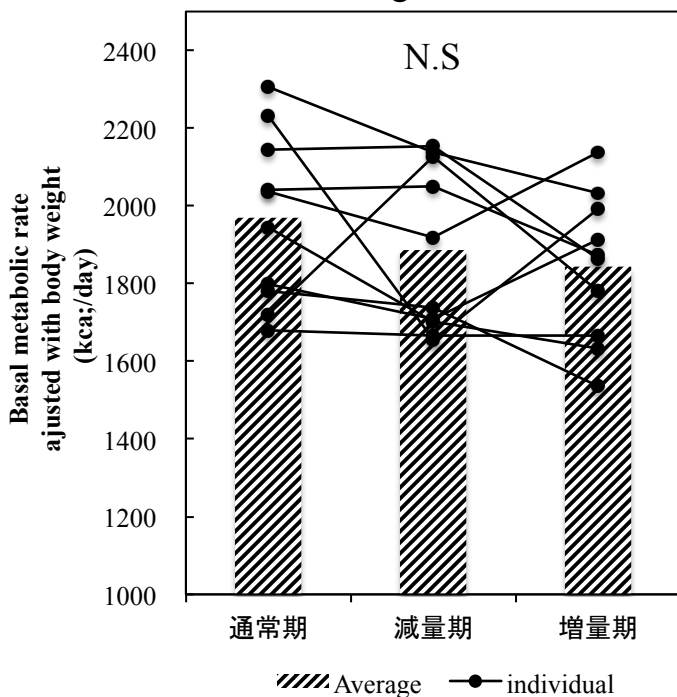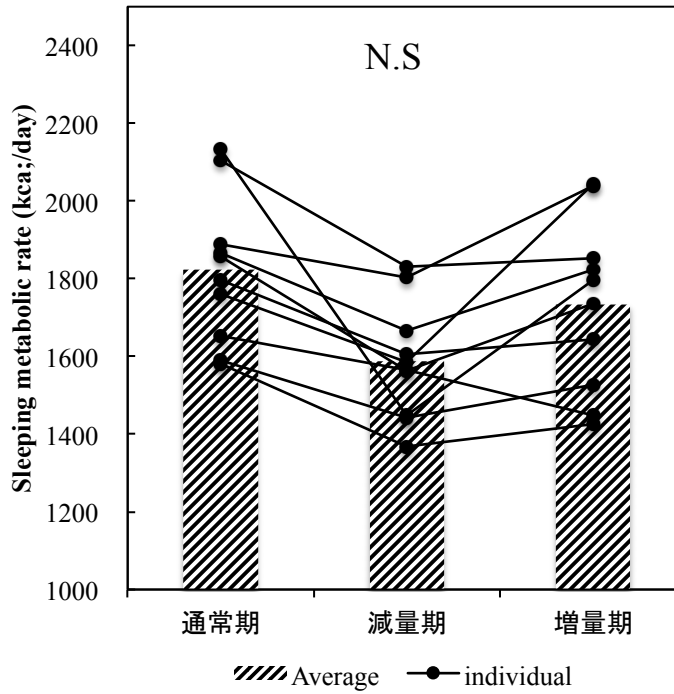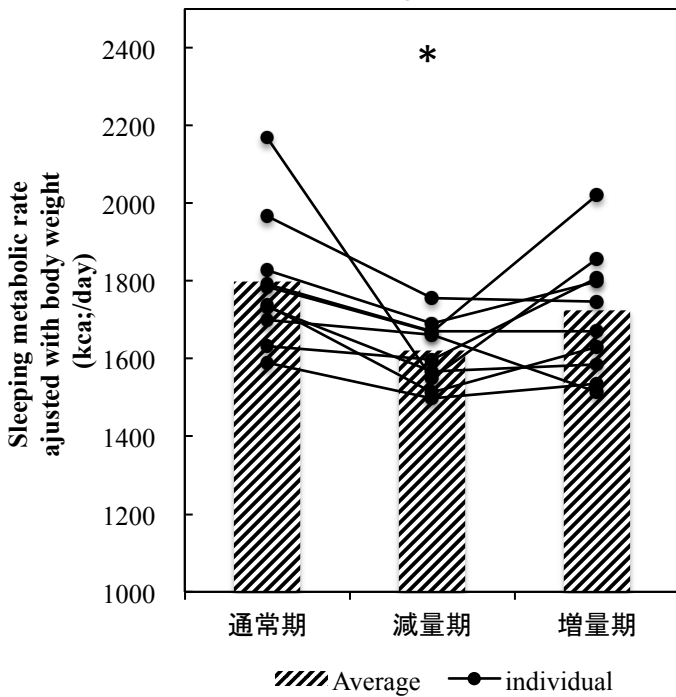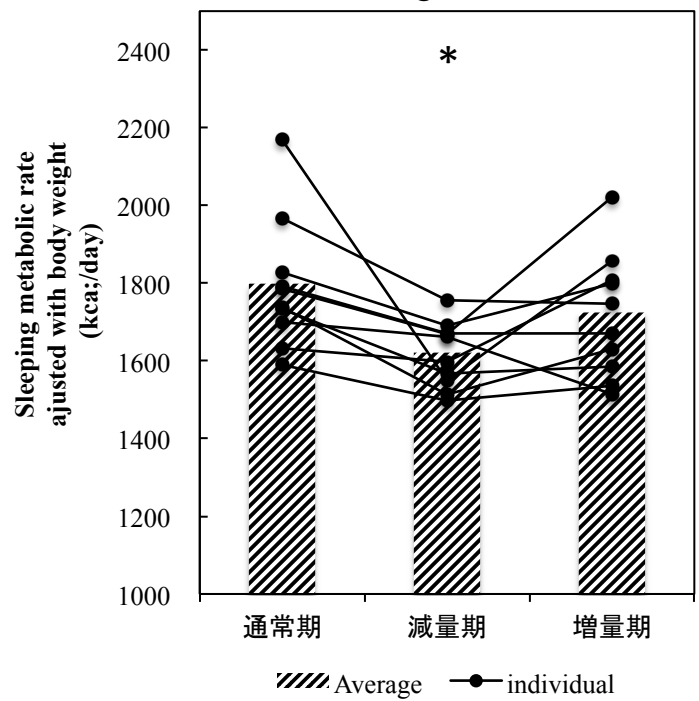[a] Estimated marginal means presented in this row is calculated by ANCOVA with weight as covariates set at 60.2 kg.

[b] Estimated marginal means presented in this row is calculated by ANCOVA with FFM as covariates set at 72.5 kg.

第七章

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第八章

副論文

ARTICLE

Effects of rapid weight loss and regain on body composition and energy expenditure

Hiroyuki Sagayama, Eiichi Yoshimura, Yosuke Yamada, Mamiko Ichikawa, Naoyuki Ebine, Yasuki Higaki, Akira Kiyonaga, and Hiroaki Tanaka

Abstract: Weight-classified athletes need an energy intake plan to accomplish target weight reduction. They have to consider body composition and energy metabolism during rapid weight loss followed by rapid weight regain to achieve their energy intake plan. We investigated the effects of rapid weight loss, followed by weight regain, on body composition and energy expenditure. Ten weight-classified athletes were instructed to reduce their body weight by 5% in 7 days. Following the weight loss, they were asked to try to regain all of their lost weight with an ad libitum diet for 12 h. Food intake was recorded during the baseline, weight loss, and regain periods. Fat mass, total body water, and fat-free dry solids were estimated by underwater weighing and stable isotope dilution methods. A three-component model was calculated using Siri's equation. Basal and sleeping metabolic rates were measured by indirect calorimetry. Body composition and energy expenditure were measured before and after weight loss and after weight regain. Body weight, total body water, and fat-free dry solids were decreased after the weight loss period but recovered after weight regain ($p < 0.05$). Basal metabolic rate did not change throughout the study. Sleeping metabolic rate decreased significantly during weight loss but recovered after weight regain. Changes in total body water greatly affect body weight during rapid weight loss and regain. In addition, rapid weight loss and regain did not greatly affect the basal metabolic rate in weight-classified athletes.

Key words: rapid weight loss, rapid weight regain, energy metabolism, body composition, total body water, three-component model, metabolic chamber.

Résumé : Les athlètes classés selon le poids corporel ont besoin d'un programme d'apport énergétique pour perdre le poids voulu. Ce programme doit prendre en compte la composition corporelle et le métabolisme énergétique durant la période de perte accélérée de poids suivie d'une période de gain accéléré de poids. Dans cette étude, nous examinons les effets de la perte rapide de poids suivie du gain de poids sur la composition corporelle et la dépense énergétique. On demande à dix athlètes classés selon le poids de diminuer de 5 % leur masse corporelle en 7 jours. Après cette période, on leur demande de reprendre tout le poids perdu en suivant un régime ad libitum durant 12 h. On enregistre l'apport alimentaire durant les périodes de référence, de perte de poids et de reprise du poids. On évalue la masse adipeuse, la quantité totale d'eau corporelle et les solides secs sans gras par la méthode de la pesée hydrostatique et la méthode de dilution isotopique avec des isotopes stables. On élabore un modèle à trois compartiments en utilisant l'équation de Siri. On évalue le métabolisme de base et le métabolisme du sommeil par calorimétrie indirecte. On détermine la composition corporelle et le métabolisme énergétique avant et après la perte de poids et après la reprise du poids. La masse corporelle, la quantité totale d'eau corporelle et les solides secs sans gras diminuent durant la période de perte de poids, mais reviennent à la normale après la période de reprise ($p < 0,05$). Le métabolisme de base ne varie pas tout au long de l'étude. Le métabolisme du sommeil diminue significativement durant la perte de poids, mais revient à la normale après la reprise de poids. Les variations de la quantité totale d'eau corporelle affectent grandement la masse corporelle durant la perte et la reprise du poids. De plus, la rapidité de la perte et de la reprise du poids n'affecte pas beaucoup le métabolisme de base chez les athlètes classés selon le poids. [Traduit par la Rédaction]

Mots-clés : perte rapide de poids, reprise rapide du poids, métabolisme énergétique, composition corporelle, eau corporelle totale, modèle à trois compartiments, enceinte métabolique.

Introduction

Weight-classified sports such as judo, wrestling, and boxing apply strict weight limits, forcing athletes to go on stringent weight loss diets. Many athletes in weight-classified sports enter competitions in a lower weight class than their normal weight. Thus, athletes tend to have periods of rapid weight loss to achieve their target weight within a period of approximately 1 week, followed by rapid weight gain to regain the weight within a couple hours to a day (Brito et al. 2012; Choma et al. 1998; Kiningham and Gorenflo 2001; Oppliger et al. 1998, 2003, 2006; Steen and Brownell 1990).

This kind of rapid weight loss and regain is likely to cause changes to the athlete's body composition. Several previous studies have investigated the changes in body composition during

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rapid weight loss using methods such as skinfold thickness measurements and bioelectrical impedance analysis (BIA), which are secondary indirect methods (Dixon et al. 2005, 2006; Kordi et al. 2012; Rankin et al. 1996). However, these methods rely on several assumptions, and it is unknown if changes in body composition are accurately reflected when there are sudden changes such as rapid weight loss.

Primary indirect methods of estimating body composition include underwater weighing (UWW) measurement, stable isotope dilution methods, dual-energy X-ray energy absorptiometry (DXA), and magnetic resonance imaging (MRI). However, these methods still rely on several assumptions, and the percentage of body fat cannot be accurately measured with a single measurement alone under conditions in which the percentage of fat-free mass (FFM) may change such as during rapid weight loss. Accordingly, three-component models that combine UWW measurement and stable isotope dilution methods and four-component models that have the addition of DXA are used as the gold standard for estimating the percentage of body fat. The variance in bone mineral content using DXA during rapid weight loss is less than 100 g and does not have a large effect in estimating fat mass (FM) and FFM (Karila et al. 2008). Therefore, the three-component model, which consists of FM, total body water (TBW), and fat-free dry solids (FFDS), also has high validity for clarifying changes in FM and FFM during rapid weight loss. However, there are no reports evaluating changes in the three-component model during rapid weight loss and the subsequent rapid weight regain in weight-classified athletes. There is potential for changes in TBW by rapid weight loss because of liquid restriction, dehydration, and reduction in glycogen (Tarnopolsky et al. 1996). Therefore, we assumed that rapid weight loss and regain has a short-term influence on the three-component model, in particular TBW.

Additionally, there is a high correlation between skeletal muscle mass and energy expenditure (EE) at rest, e.g., the basal and sleeping metabolic rates (BMR and SMR, respectively) (Ganpule et al. 2007; Santos et al. 2010). A decrease in FFM may affect these parameters. A previous study in wrestlers who achieved rapid weight loss of 2.9 kg in 1 week reported that BMR and SMR per kilogram of FFM decreased significantly (Kukidome et al. 2007). However, the TBW and FFM in this study were estimated by BIA, which has low validity, as mentioned above; thus, it is not known whether the decrease in FFM is the result of a decrease in TBW or in FFDS. Therefore, we hypothesize that if the decrease of TBW in FFM is the main component in the decrease of body weight by rapid weight loss, then TBW and FFM could recover by weight regain, and BMR and SMR might not change.

Thus, the primary purpose of the present study was to evaluate the changes in FM and FFM during rapid weight loss and subsequent weight regain using the three-component model of UWW measurement and stable isotope dilution. The second purpose of our study was to clarify the effects of short-term rapid weight loss and rapid weight regain on BMR and SMR. The guidelines of the American College of Sports Medicine and the National College Athletic Association require assessment of body composition for the competitive season (Clark et al. 2007). The results of the present study should help reinforce the guidelines.

Materials and methods

Study subjects

Data were obtained from 10 healthy Japanese male weight-classified athletes (7 boxers and 3 judokas; mean age = 20.0 ± 1.4 years). The subjects were invited to attend an information meeting and those interested in participating provided written informed consent. The Ethics Committee of Fukuoka University approved the study protocol.

Experimental design

Three time points were evaluated: the day before 1 week of weight loss (baseline (BL)), the day after 1 week of weight loss (weight loss (WL)), and the day after the WL measurement, when body weight had recovered (weight regain (WR)). Subjects were asked to lose 5% of their BL body weight in 1 week by self-selected means. The self-selected means were at the discretion of each individual; however, post hoc investigation revealed energy and fluid restriction, increasing volume of exercise, and dehydration methods (i.e., sauna, exercising with heating clothes). However, the use of laxatives, diuretics, or diet pills was an exclusion criterion for ethical reasons. Subjects re-fed and rehydrated ad libitum for 12 h after the WL period. Following the WL period, subjects were asked to try to regain all of their lost weight with an ad libitum diet for 12 h (Fig. 1).

Body composition measurements

The subjects came to the laboratory early in the morning of the BL, WL, and WR measurement days after a 12 h fast. They had refrained from exercise, alcohol, or stimulant beverages for at least 24 h. All body composition measurements were carried out at the same time each morning after urination and defecation. Height was measured to the nearest 0.1 cm with a stadiometer. Body weight was measured using a calibrated balance beam scale (Shinko Denshi Vibra Co., Ltd., Tokyo, Japan) to the nearest 0.01 kg with the subjects wearing only light undergarments. Body density was estimated by UWW with correction for the residual lung volume measured by simultaneous O_2 re-breathing.

TBW was measured using the ^2H and ^{18}O dilution method. A BL urine sample was obtained and the participants drank either ~0.12 g of ^2H_2O diluted 20-fold (^2H_2O 99.8 atom%; Taiyo Nippon Sanso, Tokyo, Japan) or ~2.5 g of $H_2^{18}O$ diluted 20-fold ($H_2^{18}O$ 20.0 atom%; Taiyo Nippon Sanso) per kilogram of their predicted TBW. The body fat percentage (%fat) was assumed to be 15%, and TBW was predicted as body weight × 0.85 × 0.732. Urine samples were collected 2, 3, and 4 h after dosing (Yamada et al. 2009a). BL samples were collected on each study day. The 3 and 4 h urine samples were averaged for the TBW calculations. TBW was measured on two consecutive days (the day after WL and the day after the WL measurement, when body weight had recovered). If the same isotope is used on 2 consecutive days, the concentration of isotope in the body on the second day can be expected to be very high. Therefore, we decided to measure TBW using two different stable isotopes on the 2 days. The subjects drank either ^2H_2O or $H_2^{18}O$ at one time point in the TBW measurements. Subjects were randomly assigned to the following two protocols: ^2H_2O, $H_2^{18}O$, and ^2H_2O for BL, WL, and WR, respectively, or $H_2^{18}O$, ^2H_2O, and $H_2^{18}O$ for BL, WL, and WR, respectively. Before the study, we conducted a pilot study in which 10 healthy young male adults participated. Participants drank ^2H_2O on the first day and $H_2^{18}O$ on the second day. The TBWs calculated by ^2H_2O and $H_2^{18}O$ were compared. There was no significant difference between the two methods (38.7 ± 2.1 vs. 38.7 ± 2.0 kg, $p = 0.562$), and high correlation was observed ($r = 0.984$).

Aliquots of urine were frozen at −30 °C for later isotope ratio mass spectrometry analysis (SerCon Isotope Ratio Mass Spectrometers CF 20-20; Sercon Ltd., Crewe, UK). The equilibration gas for ^{18}O was CO_2 and that for ^2H was H_2. A Pt catalyst was used to equilibrate ^2H. The enrichment of equilibrated local water standards was calibrated against the standard mean ocean water standards. Samples and the corresponding references were analyzed in triplicate. The ^2H and ^{18}O dilution spaces (N_d and N_o, respectively) were determined by dividing by the dose of administered tracer (as moles of ^2H or ^{18}O water). TBW was calculated as N_d divided by 1.041 for the dilution space measured by ^2H, and N_o was divided by 1.007 for the dilution space measured by ^{18}O (Racette et al. 1994).

Fig. 1. Study protocol for weight loss and regain.
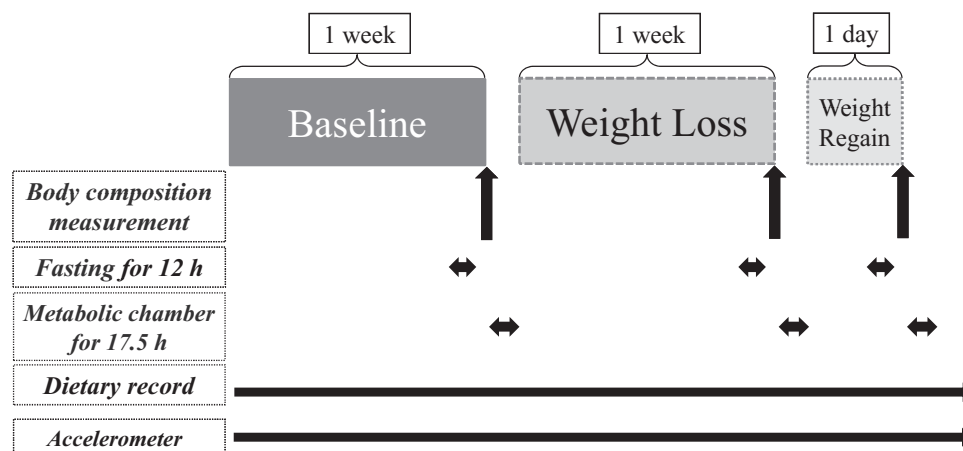
Three-component model

The %fat was estimated using Siri's three-component model formula (Siri 1993): %fat = (2.118/body density − 0.78 × TBW − 1.354) × 100. FFDS were calculated by subtracting FM and TBW from the body weight. FFM was the sum of TBW and FFDS.

Physical activity level and energy intake

Daily activity energy expenditure was evaluated using a triaxial accelerometer (Panasonic Electric Works Co., Ltd., Osaka, Japan) (Yamada et al. 2009b), which was attached to the participant's waist for 16 days until the end of the study. Data were averaged for each period. If the activity time during which the accelerometer was not worn exceeded 3 h in a day with the exception of practice, training, taking a bath, and sleeping, that day was excluded from the analysis. The EE of practice and training was estimated from the kind of training and a MET values chart (Ainsworth et al. 2011). The physical activity level (PAL) was calculated by adding daily activity EE and the EE of practice and training. Subjects were instructed to refrain from any practice and training during the 1-day weight regain period.

A survey of food intake was conducted using both self-reporting methods and visual records obtained using a digital camera or a mobile phone with a camera as described previously (Kukidome et al. 2007). A well-trained registered dietitian calculated nutrients from the diet records with photographs. Energy intake was measured daily from 1 week before the BL measurement until the WR measurement. Total energy and macronutrient consumption were determined over a random 3-day period during the BL periods and from all food records for the WL and WR periods. All diet records were analyzed using a computerized nutrient analysis program (Excel Eiyoukun Ver. 4.5; Kenpakusha, Tokyo, Japan).

Metabolic chamber

An open-circuit indirect metabolic chamber (MC) was used to evaluate the 7 h SMR and 30 min BMR. The respiratory chamber was an airtight room (19 500 L) equipped with a bed, desk, chair, TV, telephone, toilet, and sink. The temperature was controlled at 25 °C and the relative humidity was 50%. The room air was sufficiently circulated with fans. The oxygen and carbon dioxide concentrations of the air supply and exhaust were measured with high precision by online process mass spectrometry (VG Prima δB; Thermo Fisher Scientific, Cheshire, UK). The gas analyzer was calibrated for each experiment using four-type gas calibration bottles.

Oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) were determined from the rate of exhaust from the chamber and the O_2 concentration in the inlet air or the CO_2 concentration in the outlet air of the chamber using Henning's algorithm

Table 1. Timetable for the modeling day in the metabolic chamber (MC).

Time (hours)	Action
0630	Arrive for body composition measurement
0640	Urine sample collection at baseline
0650	Administer stable isotope
0700	Underwater weighing
0850	Urine sample (2 h)
0950	Urine sample (3 h)
1050	Urine sample (4 h)
1700	Entry into MC
1740	Dinner (self-selected diet)
1800	Sit quietly
2300	Go to sleep → SMR (2300–0600)
0600	Wake up → BMR (0600–0630)
0630	Get up
0640	Breakfast (prescribed diet)
0700	Sit quietly
1030	Exit from MC

Note: BMR, basal metabolic rate; SMR, sleeping metabolic rate.

(Henning et al. 1996). EE (kcal·min⁻¹) was determined from the $\dot{V}O_2$ and $\dot{V}CO_2$ using Weir's equation (Weir 1949).

The accuracy and precision of our MC for measuring $\dot{V}O_2$ and $\dot{V}CO_2$ as determined by the alcohol combustion test at 3 h were 99.97% and 100.03%, respectively.

Design for timetables in the MC

Table 1 shows the timetable for the measurement day in the MC. All subjects abstained from intense exercise during the 24 h preceding their assessment day to circumvent the effect of excess postexercise oxygen consumption. Except for using the toilet, the subjects were permitted to carry out only light activities such as reading, writing, and watching TV in a sitting position. Sleeping was not permitted until going to bed. The same breakfast was provided during each period.

Statistical analysis

All analyses were performed using PASW Statistics for Windows, Version 18.0 (SPSS Inc., Chicago, Illinois). The results are presented as means ± standard deviations. An alpha of 0.05 was used to denote statistical significance. Data comparisons were conducted using a one-way repeated-measures analysis of variance (ANOVA). To eliminate the effect of decreasing weight and FFM on BMR or SMR, repeated-measures analysis of covariance (ANCOVA) was conducted to compare BMR or SMR between BL, WL, and WR with weight or FFM as a covariate. When a significant

Table 2. Changes in body composition in the three-component model.

	Baseline	Weight loss	Weight regain
Age (years)	20.0±1.4	—	—
Height (cm)	174.6±6.8	—	—
Body weight (kg)	74.4±9.0	70.0±8.8* (−4.4±0.6)	73.1±8.6*# (−1.3±0.8)
% Fat	17.0±7.4	15.8±7.6* (−1.2±1.0)	15.9±8.2* (−1.1±1.2)
FM (kg)	13.1±7.3	11.7±7.5* (−1.5±0.6)	12.1±7.3*# (−1.1±0.8)
FFM (kg)	61.2±4.2	58.3±3.8* (−2.9±0.8)	61.1±3.7# (−0.2±0.9)
TBW (kg)	43.8±3.1	41.5±2.9* (−2.3±0.9)	43.6±2.6# (−0.2±1.0)
FFDS (kg)	17.5±1.2	16.8±1.1* (−0.7±0.3)	17.5±1.2# (0.0±0.3)

Note: Values are means ± standard deviations (n = 10). The case arc is the amount of change vs. baseline. Significance: *, $p < 0.05$ vs. baseline; #, $p < 0.05$ vs. weight loss. FM, fat mass; FFM, fat-free mass; TBW, total body water; FFDS, fat-free dry solids. FFM shows the sum of TBW and FFDS.

difference was detected, a multiple comparison test was performed using Tukey's post-hoc test for least significant difference.

Results

Body composition

Body weight significantly decreased between BL and WL (−6.0% ± 0.9%) (Table 2). Body weight had increased again by WR but remained lower than at BL. TBW significantly decreased between BL and WL (−5.1% ± 1.8%) and was back to BL values at WR. FFDS significantly decreased between WL and BL (−3.9% ± 1.5%) and was back to BL values at WR. FM significantly decreased between BL and WL (−15.5% ± 12.1%). FM had increased again by WR but remained lower than at BL (−10.0% ± 6.5%). The percentage of FFM contributing to rapid weight loss was 66.2%. The percentage of FFM contributing to rapid weight regain was 90.8%.

Energy expenditure

The BMR tended to decrease between BL and WL, but this was not significant (p = 0.09). No significant changes were observed in BMR adjusted by weight or FFM throughout the study (Table 3). The SMR significantly decreased between BL and WL but returned to BL values at WR. The SMR adjusted by body weight decreased between BL and WL but returned to BL values at WR because of the subsequent rapid weight regain (Table 3). The SMR adjusted by FFM tended to decrease between BL and WL, but this was not significant (p = 0.07).

Physical activity level and energy intake

Energy intakes significantly decreased between BL and WL and were significantly increased at WR (Table 4). Protein, fat, and carbohydrate intakes significantly decreased between BL and WL and were significantly increased at WR. The percentages of the total energy intake made up by the different macronutrients are shown in Table 4. The percentage of energy intake made up by fat was significantly higher in the WR period than in the WL period, but there were no significant differences in the energy percentage of protein and carbohydrate intake. No significant difference in PAL was observed between BL and WL. However, PAL decreased significantly from BL and WL values during the WR stage.

Discussion

The major finding of this study is that TBW and FM are the main components of typical rapid weight loss and subsequent 1-day rapid weight regain in weight-classified athletes. This study focused on changes in body composition during rapid weight loss and rapid weight regain in weight-classified athletes, particularly the declining ratio of FM to FFM and changes in TBW. Body weight decreased by 6.0% after rapid weight loss and increased by 4.6% in the weight-regaining period. FM remained lower after weight regain compared with before weight loss, probably because the athletes did not regain all of the lost weight. The percentage of FFM contributing to rapid weight

loss was 66.2%. The percentage of FFM contributing to rapid weight regain was 90.8%. The three-component model showed that 50.6% of the body weight change during weight loss was from the decline in TBW, 33.8% was from the decline in FM, and 15.6% was from the decline in FFDS. Of the weight regained following weight loss, 67.5% was from the increase in TBW, 9.2% was from the increase in FM, and 23.3% was from the increase in FFDS. In a previous study using MRI to evaluate the body composition of wrestlers, the cross-sectional area of femoral skeletal muscle decreased by 5.9% after rapid weight loss over a week. However, it had recovered to BL values in the measurement taken immediately after the competition (Kukidome et al. 2008). It was determined that the cross-sectional area of the skeletal muscle increased because of the increased food and water intakes between the weigh-in and the competition. The results of our study may update the TBW data of the previous study.

The FFDS decreased by 0.7 kg during the weight loss period in this study. FFDS is composed of glycogen, minerals, and protein. As mentioned above, bone mineral content decreases by less than 100 g during rapid weight loss (Karila et al. 2008). The molecular fraction of glycogen is hydrated with water molecules by approximately 1:3 and structurally contains abundant water (King et al. 2008). Moreover, glycogen is known to be present in skeletal muscle and the liver, and there are reports on changes in body water when glycogen fluctuates (Fenn and Haege 1940; Olsson and Saltin 1970; Puckett and Wiley 1932). The estimated glycogen content of a body weighing 80 kg with 15% body fat is 520 g, 20 g of which are in the blood cells, 100 g in the liver, and 400 g in skeletal muscle (Jeukendrup 2003). If skeletal muscle glycogen is hydrated with water molecules by approximately 1:3, approximately 1.2 kg of TBW is stored. A study of a group of wrestlers reported that although muscle glycogen decreased by 54% after rapid weight loss, no changes in muscle glycogen were observed during weight regain in the 17 h following weight loss (Tarnopolsky et al. 1996). This suggests that there is a 0.648 kg decrease in glycogen in skeletal muscle, which is similar to the 0.7 kg decline in FFDS at a body weight of 74.4 kg in our study. Considering that TBW largely contributed to the weight loss and regain in our study, it may be surmised that the change in glycogen is involved in the change in TBW, whereas the decline in FFDS is related to the decline in glycogen.

FM decreased by an average of 1.5 kg during the WL. The mean total energy expenditure during the WL evaluated from the PAL and BMR was 3490 ± 560 kcal·day⁻¹, whereas the mean total energy intake evaluated from the dietary record was 1008 ± 354 kcal·day⁻¹. The calculated decrease in FM from these data is 1.89 kg, which is close to the true decrease found. Thus, the decrease in FM is the result of a negative energy balance, which illustrates the effectiveness of 1 week of rapid weight loss. Weight loss during the WL may also be caused by increased physical activity. However, we found no difference in PAL between the BL and

Table 3. Changes in energy expenditure.

	Baseline	Weight loss	Weight regain
BMR (kcal·day⁻¹)	1998±242	1846±261[†]	1852±243
BMR adjusted by weight (kcal·day⁻¹)[a]	1969±220	1885±213	1843±190
BMR adjusted by FFM (kcal·day⁻¹)[b]	1945±132	1943±209	1809±79
SMR (kcal·day⁻¹)	1822±191	1587±150*	1734±222[#]
SMR adjusted by weight (kcal·day⁻¹)[a]	1798±169	1620±88*	1725±179
SMR adjusted by FFM (kcal·day⁻¹)[b]	1786±100	1654±116[‡]	1704±169
DIT (kcal·h⁻¹)	23±8	11±7*	19±5[#]

Note: Values are means ± standard deviations ($n = 10$). Significance: *, $p < 0.05$ vs. baseline; [†], $p = 0.09$ vs. baseline; [‡], $p = 0.07$ vs. baseline; [#], $p < 0.05$ vs. weight loss. BMR, basal metabolic rate; SMR, sleeping metabolic rate; FFM, fat-free mass (including total body water); DIT, diet-induced thermogenesis. DIT was obtained from the difference in the postprandial resting metabolic rate and BMR for 3 h at one meal.

[a]Estimated marginal means presented in this row are calculated by ANCOVA, with weight as covariate set at 72.5 kg.

[b]Estimated marginal means presented in this row are calculated by ANCOVA, with FFM as covariate set at 60.2 kg.

Table 4. Energy intake and physical activity level (PAL).

	Baseline	Weight loss	Weight regain
Energy intake (kcal·day⁻¹)	2458±578	1008±354*	3066±506*[#]
Protein			
g·day⁻¹	86±22	31±10*	101±19[#]
g·kg⁻¹·day⁻¹	1.17±0.33	0.45±0.15*	1.39±0.24[#]
%[a]	14±1	12±3	13±2
Fat			
g·day⁻¹	79±24	28±13*	113±24*[#]
%[a]	29±5	23±9	32±6[#]
Carbohydrate			
g·day⁻¹	334±88	148±57*	396±81*[#]
%[a]	55±5	58±15	53±8[†]
PAL	1.88±0.13	1.93±0.14	1.40±0.17*[#]

Note: Values are means ± standard deviations ($n = 10$). Significance: *, $p < 0.05$ vs. baseline; [#], $p < 0.05$ vs. weight loss; [†], $p = 0.05$ vs. weight loss. PAL, physical activity level.

[a]Percentage of the total energy intake.

the WL. This suggests that the weight loss in this study was mainly the result of a decrease in FM due to dietary restrictions.

The emptying time of a solid meal from the normal stomach may be as little as 3 h and should never exceed 6 h (Rose 1979). Because the UWW measurements were conducted after 12 h of fasting at each point, one can assume that the gastric content was negligible at all three measurement points. However, total transit time, from eating to elimination in stool, averaged 53 h (24 to 72 h) (Metcalf et al. 1987). Thus, the bowel content should be different between conditions: the amount of bowel content is as usual at BL, remarkably smaller after rapid weight loss, and larger after rapid weight regain (Tai et al. 2010). Stool normally contains 75% water. When measured directly, water content is 82%–83% in the transverse colon, 84%–85% in the right colon, and 90%–95% in the ileocecum (Jensen et al. 1976; Goiffon et al. 1961). Thus, most of the bowel content is water and the hydration factor is greater than 0.732. The larger amount of bowel contents will slightly overestimate the %fat measure from UWW. The error is in the order of 0.2% to 0.4% fat on a whole-body basis. The true %fat may be slightly lower after rapid weight loss and slightly higher after 12 h of rapid weight regain than the measured values. However, these effects are relatively small when the three-conponent model is applied to obtain %fat. It is also hypothesized that the volume of air trapped in the gastrointestinal tract is 100 mL to calculate body density from UWW. This may be affected by the condition of the bowel content, but the effect is negligible.

We found no significant changes in BMR among the study periods. It is reported that the BMR decreases with the FFM during long-term weight loss caused by dietary restrictions (Blackburn et al. 1989). In weight-classified athletes, the resting metabolic rate decreases with weight loss in the same manner (Kukidome

et al. 2007; Melby et al. 1990). This suggests that the decrease in FFM contributes to the decrease in the resting metabolic rate in these studies. However, because BIA was used for the evaluation of body composition in these studies, it is possible that the changes were not accurately measured. During weight loss in obese patients, the ratio of FM to FFM decreases at a constant ratio, with the decrease in FFM possibly affecting the BMR (Forbes 2000). The decrease in FFM over a short period of time was greater in the athletes in this study, with the ratio of FM to FFM being approximately 1:2. However, this study is different from prior studies because most of the decrease in FFM was caused by changes in TBW, suggesting that the BMR was not affected by rapid weight loss and (or) rapid weight regain.

Unlike the BMR, the SMR significantly decreased during WL (−235 kcal·day⁻¹). It has been reported that the SMR decreases after fasting for 48 h (Weyer et al. 2001) and that diet-induced thermogenesis (DIT) decreases with dietary restrictions and (or) weight loss (Platte et al. 1996). In the present study, food intake during the weight loss period averaged 1008 ± 354 kcal·day⁻¹. Thus the decrease in SMR may have been caused by the strict dietary restrictions during the WL period. It has been reported that DIT continues for 6 h or more after meals (Westerterp 2004), and there may be little effect on the BMR within 12 h after a meal, although the SMR is heavily affected 5 h after a meal. These may be contributing factors in the difference between the BMR and SMR results. In this study, DIT was measured 3 h after a meal in a MC. DIT decreased by approximately 49% in the WL compared with BL, and the possibility that the effect of DIT remained in the BL and the sleeping hours during the WR cannot be ruled out. Moreover, a study that investigated the difference in energy expenditure of the BMR and SMR shows that the comparison of the SMR and the BMR has a margin of error of approximately 1% at 8 h (Ganpule et al. 2007). The SMR only approximates the BMR. In a study in Japanese people by Kumahara et al. (2004), the SMR–BMR ratio in men was 93%. The SMR–BMR ratio in the present study was 91.2% at BL and 93.6% during the WR, while a much lower value (86.0%) was found during the WL. This also suggests an effect of a decrease in DIT during the WL.

It is believed that the weight loss experience, the number of times of weight loss, and weight cycling may affect EE (Horswill 1993). In this study, the effects of weight cycling were not taken into consideration. The weight loss experience, the number of times of weight loss, and the method of weight loss should be investigated via questionnaires in future studies. Moreover, the recovery period following rapid weight loss varies from several hours to a day depending on the type of sport. In this study, this was set to 1 day for a more accurate measurement of body composition and EE. In the current study, TBW had a large impact on the weight fluctuations observed during rapid weight loss and regain. However, water consumption, whole-body glycogen content, bone mineral content, and skeletal muscle protein were not

measured. Future studies need to address which components of the FFDS affect the weight fluctuations during rapid weight loss and regain in athletes. Also, these studies need to collect data related to water consumption.

In conclusion, TBW and FM are the main components of rapid weight loss and subsequent 1-day rapid weight regain in weight-classified athletes. It suggests the possibility that BMR does not significantly change in short-term rapid weight loss and regain. Moreover, it may be an effective way for weight-classified athletes to reduce and regain weight in a short time, allowing them to maintain fat-free dry solids and reduce fat mass.

Conflicts of interest

The authors state that there are no personal conflicts of interest in the present study.

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Measurement of body composition in response to a short period of overfeeding

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Abstract

Background: Obesity and overweight are increasing in prevalence in developed countries as a result of changing dietary habits and a lack of physical activity. The purpose of the present study was to evaluate the changes in body composition during short-term overfeeding using the three-component model, which is composed of fat mass (FM), total body water (TBW), and fat-free dry solids (FFDS).

Methods: Ten healthy men completed 3 days of overfeeding during which they consumed 1,500 kcal/day more energy than consumed in their normal diets. Body composition was evaluated at three time points: the day before and after their normal diets and the day after the 3-day overfeeding diet.

Results: Before and after their normal diets, there were no significant differences in body weight and composition, but after 3 days of overfeeding, body weight, TBW, and FFDS increased 0.7, 0.7, and 0.2 kg, respectively (P <0.0001). There was no significant difference in FM between the normal and overfeeding diets.

Conclusion: This study suggests that TBW gain contributes to weight gain following a short-term overfeeding.

Keywords: overfeeding, body composition, total body water

Background

Obesity and overweight are increasing in prevalence in developed countries as a result of changing dietary habits and a lack of physical activity (PA) [1-4]. Both conditions are caused by a chronic imbalance between energy intake (EI) and expenditure (EE). A positive balance between EI and EE is a key factor in weight gain caused by overfeeding or decreasing activity energy expenditure (AEE). Most of the accumulation of excess energy is stored as lipid, mainly triglycerides, with overfeeding [5]. Lipid is ideal for long-term energy store, with little water accumulation in humans. Therefore, huge quantities of triglycerides can be stored with increasing adipocyte size and number during positive energy balance [6,7].

Several previous studies have suggested that EI exceeding EE for 2 to 8 weeks led to increased fat mass (FM) [8-10]. Moreover, the concept of non-exercise activity thermogenesis (NEAT) seems important in energy balance regulation as in the study, which overfed 16 non-obese subjects with 4.2 MJ/day for 56 days; changes in NEAT directly predicted resistance to FM gain from overfeeding [8]. Additionally, there is an association between weight gain and sedentary time during 3 days of overfeeding [11]. Thus, AEE is the most important component of energy expenditure to maintain body weight and composition during overfeeding. However, there is little detailed evidence of changes in body composition when AEE is maintained during overfeeding. Additionally, there is poor information regarding body composition during short-term overfeeding. Therefore, we hypothesized that fat mass would not be gained during overfeeding if AEE could be maintained. Thus, the purpose of the present study was to evaluate changes in body composition during short-term overfeeding using the three-component model, which includes FM, total body water (TBW), and fat-free dry solids (FFDS).

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Methods

Ten healthy, non-obese Japanese men participated in this study (mean ± standard deviations; age = 23.1 ± 1.6 years; height = 171.7 ± 3.6 cm; body weight = 63.6 ± 4.5 kg; and body mass index = 21.6 ± 1.3 kg/m^2). All subjects lacked chronic diseases that could affect body composition, metabolism, or daily PA. The subjects were invited to attend an informational meeting and those interested in participating in the study provided written informed consent. The study protocol was approved by the Ethics Committee of Fukuoka University (10-12-02).

The experimental design of the study is shown in Figure 1. Body composition was evaluated at three time points: the day before the 3-day normal diet of the survey period (Baseline[1st] [BL[1st]]); the day after the 3-day normal diet of the survey period (this day is the same measurement before overfeeding) (Baseline[2nd] (BL[2nd])); and the day after the overfeeding diet period (Overfeeding (OF)). Subjects measured their own body weights twice daily for the 6 days (in the morning fasting and again before going to bed) from BL[1st] to OF. Additionally, subjects measured their own body weights (in the morning fasting) for 2 days during the postintervention observation period and for 2 weeks following completion of OF.

The normal EI survey was defined over a 3-day period (between BL[1st] and BL[2nd] measurement). The overfeeding EI survey defined a 3-day period following a normal diet (between BL[2nd] and OF measurement). We informed all subjects about their normal EI to maintain that level of EI.

During the 3-day overfeeding period, subjects were overfed with a diet supplying 1500 kcal per day more energy than the 3-day normal EI. Diets were self-selected during normal and overfeeding periods. Excess EI during the overfeeding period was selected based on the energy information shown on food packages.

Body composition measurement

Subjects came to the laboratory early in the morning on the 3 measurement days after a 12-h fast. All body composition measurements were carried out at the same time each morning after urination and defecation. Height was measured to the nearest 0.1 cm with a stadiometer. Body weight was measured using a calibrated balance beam scale (Shinko Denshi Vibra Co., Ltd., Tokyo, Japan) to the nearest 0.01 kg, with the subjects wearing only light undergarments. Hydrostatic weighing and stable isotope dilution method estimated body density and total body water. Subjects were administered these stable isotopes using the following protocol: ^2H_2O, $H_2^{18}O$, and ^2H_2O for BL[1st], BL[2nd], and OF measurements, respectively. Our previous study provides details regarding the evaluation of body composition using the three-component model [12].

Physical activity and energy intake

Daily AEE was evaluated using a triaxial accelerometer (Panasonic Electric Works Co., Ltd., Osaka, Japan) [13], which was attached to the waist for about 1 month until

Figure 1 Study protocol.
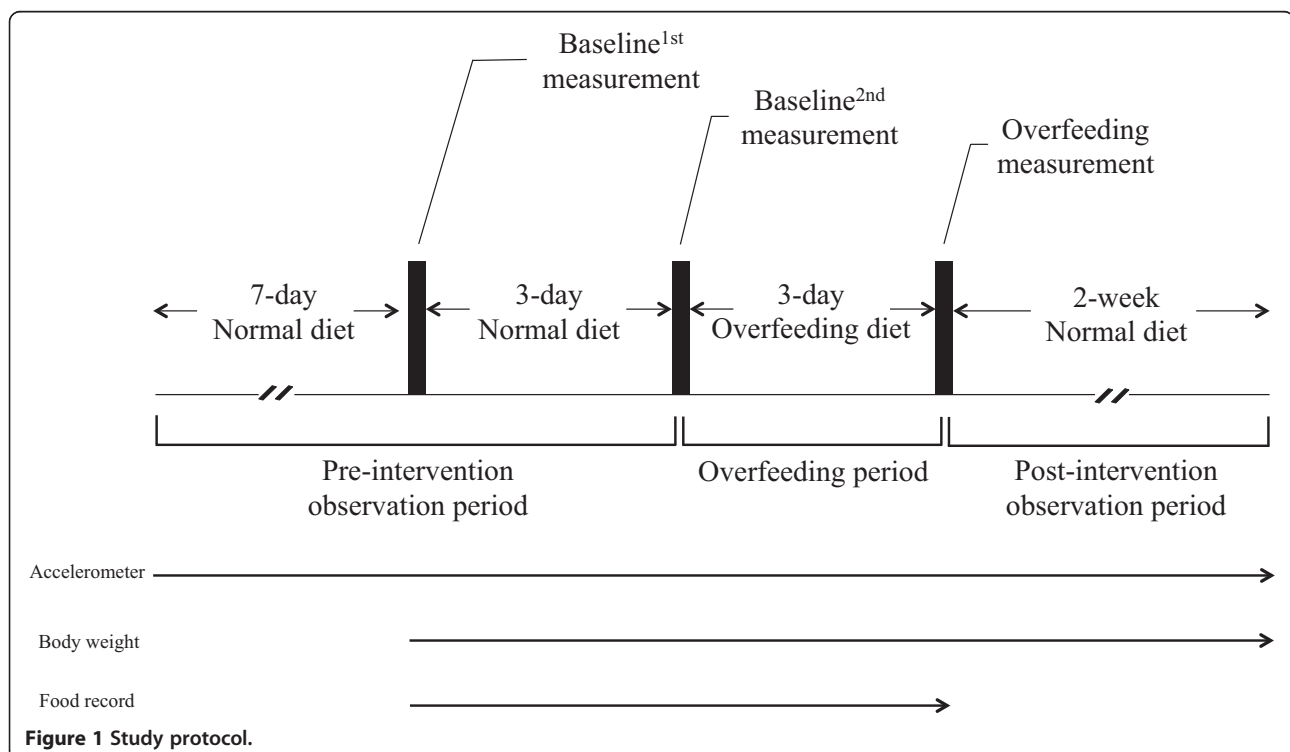
the end of the study (from 1 week before BL^{1st} until the postintervention observation period finished). Subjects were instructed to refrain from vigorous exercise and to maintain their lifestyle for about 1 month. The data of baseline PA were obtained for 7 days with the exception of the first 3 days since attaching the triaxial accelerometer. Subjects strictly maintained baseline PA by checking levels of PA using the triaxial accelerometer during the overfeeding period (between BL^{2nd} and OF measurement). If the non-wear activity time of the accelerometer exceeded 3 h in a day, with the exception of the time for taking a bath and sleeping, that day was excluded from the analysis.

All foods and beverages were weighed using a portable digital scale (KS-232; Dretec Co. Ltd., Saitama, Japan) during the BL^{2nd} and OF measurement periods (3 days). Furthermore, a survey of food intake was conducted using both self-reporting methods and visual records obtained using a digital camera or a mobile phone with a camera. A well-trained registered dietitian checked calculated nutrients from the diet records with the photographs. EI was measured daily from a week before the BL^{1st} until the OF measurement. All diet records were analyzed using a computerized nutrient analysis program (Excel Eiyoukun Ver. 4.5; Kenpakusha, Tokyo, Japan).

Statistical analysis

The results are presented as means ± standard deviations. Comparisons between two groups (BL^{1st} versus BL^{2nd} and BL^{2nd} versus OF) were made with the paired t-test using Microsoft Excel 2010 from Microsoft Office 2010 (Microsoft Corp., Redmond, WA, USA). The intraclass correlation coefficient (ICC) and the coefficient of variation (CV) were used to test the reproducibility of body weight, % fat, FM, FFDS and TBW measured by the three-component models. Values of ICC above 0.7 were considered as having excellent reproducibility. An alpha of 0.05 was used to denote statistical significance.

Results

Body compositions

We first evaluated body composition and measurement reproducibility. All components of body composition did not change between BL^{1st} and BL^{2nd} (Table 1; body weight = -0.2 ± 0.5 kg, $P = 0.17$;% fat = -0.1 ± 0.5%, $P = 0.49$; FM = -0.1 ± 0.4 kg, $P = 0.36$; TBW = -0.1 ± 0.4 kg, $P = 0.56$; FFDS = 0.0 ± 0.4 kg, $P = 0.71$). The ICC for all body composition values was above 0.9. The CV for all body composition values was less than 3%.

Body weight, TBW, and FFDS increased during OF compared with BL^{2nd} measurements (Table 2 and Figures 2, 3, and 4; body weight = 0.7 ± 0.5 kg; TBW = 0.7 ± 0.4 kg; FFDS = 0.0 ± 0.4 kg, $P < 0.0001$). There were no significant differences in FM and % fat between the BL^{2nd} and OF

Table 1 Change in body composition, coefficient of variation and intraclass correlation coefficient during normal diet

	Baseline^{1st}	Baseline^{2nd}	CV (%)	ICC
Body weight (kg)	63.6 ± 4.5	63.4 ± 4.1	0.5	0.996
Percent of fat (%)	14.5 ± 3.0	14.4 ± 3.0	2.2	0.991
Fat mass (kg)	9.3 ± 2.5	9.2 ± 2.5	2.2	0.994
Fat-free dry solid (kg)	15.5 ± 0.8	15.5 ± 0.7	1.3	0.946
Total body water (kg)	38.7 ± 2.1	38.7 ± 2.0	0.6	0.992

There were no significant differences between BL^{1st} and BL^{2nd} for each of these. CV, coefficient of variation; ICC, intraclass correlation coefficient.

measurements (Table 2). Subjects measured their body weights during the postintervention period. All subjects returned to BL^{2nd} body weights within 2 weeks (5.0 ± 4.9 days).

Physical activity and energy intake

During the normal and overfeeding periods, there were no significant differences in levels of PA (1.6 ± 0.2 and 1.6 ± 0.1, respectively) and AEE (835 ± 261 and 875 ± 240 kcal/day, respectively) (Table 3). Energy, weights of diets, and sodium intakes increased during the overfeeding period ($P < 0.05$). Fat intake significantly increased and carbohydrate intake decreased during overfeeding, affecting the protein, fat, and carbohydrate rate (PFC rate). There was no significant increase in the protein rate.

Discussion

The major finding of this study is that TBW is the main component of body composition affected during overfeeding when AEE is maintained at the level during normal diets. Our results suggested that the increased body weight for 3 days of overfeeding was mostly TBW. There were no significant differences in body weight or composition at BL^{1st} and BL^{2nd}. The ICC values ranged from 0.946 to 0.996 in the body composition measurements in the current study, which is in agreement with previous studies [14]. Thus, the results and methods are thought to be of excellent reproducibility.

The overfeeding of 1,500 kcal per day over 3 consecutive days led to increased body weight, TBW, and FFDS, though there were no significant increases in FM and %

Table 2 Changes in body composition during overfeeding

	Baseline^{2nd}	Overfeeding
Body weight (kg)	63.4 ± 4.1	64.1 ± 4.3**
Percent of fat (%)	14.4 ± 3.0	13.9 ± 3.3
Fat mass (kg)	9.2 ± 2.5	9.0 ± 2.7
Fat-free dry solid (kg)	15.5 ± 0.7	15.7 ± 0.7*
Total body water (kg)	38.7 ± 2.0	39.4 ± 1.9**

**$P < 0.01$, *$P < 0.05$ versus Baseline^{2nd} measurement.

Figure 2 Changes in body weight. *P <0.01 versus Baseline[2nd] measurement.

Figure 4 Changes in total body water. *P <0.01 versus Baseline[2nd] measurement.
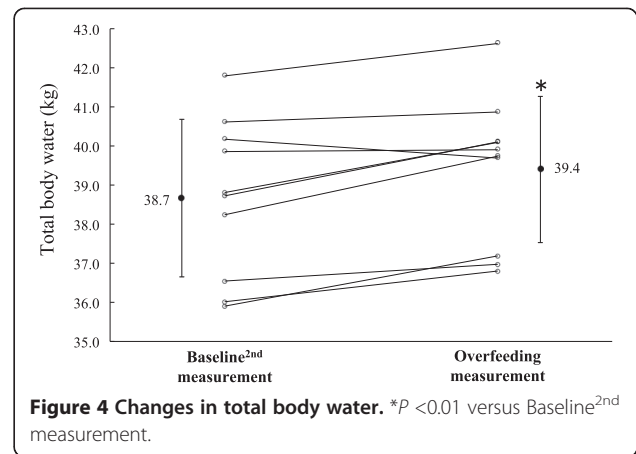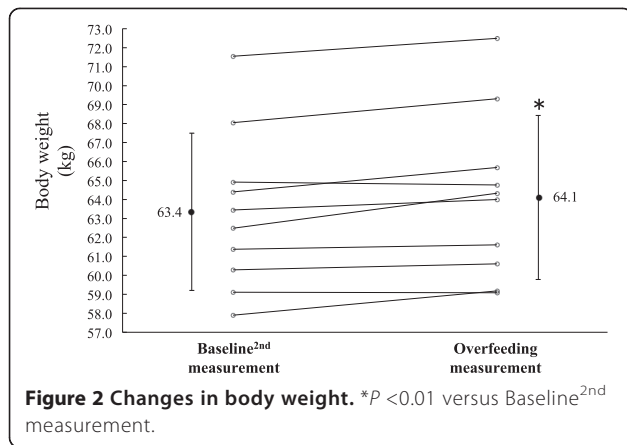
fat. Participants were asked to overeat an average of 4,500 kcal for 3 days, and were able to do so successfully. Assuming that an FM of 1 kg is equivalent to 7,000 kcal and that 85% of the EI would be accumulated as fat in this case, FM was expected to increase by 0.5 kg. However, FM did not increase. In a previous study of overfeeding an excess of 1,000 kcal per day for 8 weeks, increases in body weight, and FM were reported (weight, 1.4 to 7.2 kg; FM, 0.36 to 4.23 kg) [8]. Assuming that an FM of 1 kg is equivalent to 7,000 kcal and that 85% of the EI would be accumulated as fat in this case, the FM was expected to increase by 6.8 kg. Unexpectedly, body weight and FM in the previous study were not increased as much as expected. Moreover, there were large individual differences in the increases in FM and body weights, as pointed out by some researchers. In particular, the study suggested individual NEAT and sedentary time were different during overfeeding [8,11]. We, therefore, instructed subjects in the present study to maintain PA during overfeeding. As a result, the AEE during the 3-day overfeeding period is similar to the AEE during the normal diet period. Thus, PA is not the only factor involved in the lower-than-

expected increase in FM during overfeeding. Other factors could include an increase in diet-induced thermogenesis [15] and increased lipid catabolism [16]. The unexpected large interindividual variation in the efficiency of weight gain with overfeeding shows that adaptive thermogenesis and other factors are still an issue. Further, the accelerometers worn at the waist may not be able to evaluate arm and leg movement as a component of activity.

Body weight (on average 0.7 kg) increased as well as TBW (on average 0.7 kg) during the 3 days of overfeeding. Increased TBW could be the result of ingestion of an excess amount of sodium during overfeeding. After the ingestion of dietary sodium, there is a subsequent rise in plasma sodium, and to maintain fluid homeostasis thirst is stimulated, which promotes fluid consumption [17]. In a previous study that compared a high and low salt diet over 50 days, the high-salt diet group had a greater increase in weight compared with the low-salt group [18]. Moreover, dietary sodium is positively associated

Figure 3 Changes in fat mass.
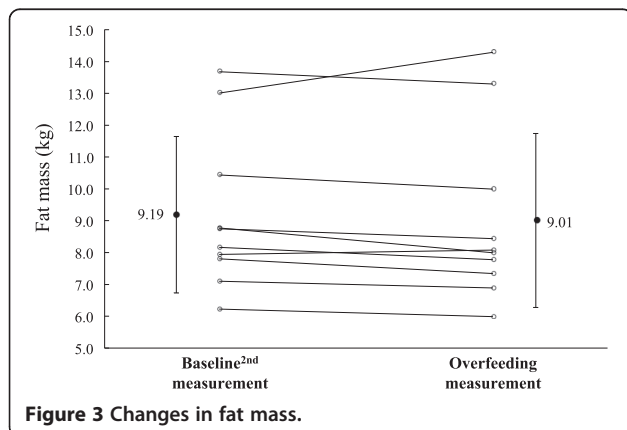
Table 3 Changes in physical activity and energy intake before and after overfeeding

	Baseline[2nd]	Overfeeding
Energy intake (kcal/day)	2452 ± 297	3906 ± 427[**]
Protein intake (kcal/day)	296 ± 44	450 ± 73[**]
Fat intake (kcal/day)	695 ± 96	1379 ± 258[**]
Carbohydrate intake (kcal/day)	1421 ± 262	2004 ± 288[**]
Protein rate (%)	12.2 ± 1.7	11.5 ± 1.1
Fat rate (%)	28.1 ± 4.4	35.3 ± 5.1[**]
Carbohydrate rate (%)	58.0 ± 4.9	51.4 ± 5.2[**]
Weight of diet (g/day)	2557 ± 615	3353 ± 724[**]
Sodium intake (mg/day)	4131 ± 1013	6079 ± 1856[**]
Physical activity level	1.6 ± 0.2	1.6 ± 0.1
Active energy expenditure (kcal/day)	835 ± 261	875 ± 240

[**]P <0.01 versus Baseline[2nd] measurement.

with fluid consumption and predicted sugar-sweetened beverage consumption [19]. Following the increase in EI, sodium intake and TBW increased in our study. Thus, water and sugar-sweetened beverage intake could be associated with these increases. The temporary accumulation of sodium may result in increased body weight as a result of transient overfeeding. Glycogen storage, which is known to increase body weight during carbohydrate overfeeding [20], may be another factor to consider. The molecular fraction of glycogen is hydrated by water molecules in a ratio of approximately 1:3, and structurally contains an abundant amount of water [21-24]. Therefore, it has the possibility to contribute to the increase seen in TBW.

The content of the diet was self-selected during the normal and overfeeding periods of our study. The EI of macronutrients during that period significantly increased in terms of PFC. However, the PFC rate was only significantly increased in terms of fat intake. These results suggested that it is possible to consume more energy from fat during self-selected overfeeding. In a previous study comparing overfeeding of high carbohydrate and high fat diets of equal energy, body weight and FM significantly increased in both diet groups. However, that study did not detect a between-group difference [9]. If self-selected overfeeding for 3 days involves a high carbohydrate diet, this may result in the promotion of more weight gain because of increased storage of glycogen and water.

The increased body weight returned to the baseline body weight over an average of 5 days, though there were individual differences (0 to 14 days). When subjects were free to follow their regular lifestyles during the postintervention period, their body weights reduced relatively early. These results support the hypothesis that the component of increased body weight in our study was a result of increased TBW.

A limitation of our study is that a diet survey and information regarding bowel movements were not measured during the postintervention period. The EI during the postintervention observation period is a matter of speculation; differences in each subjects' EIs were considered a possible effect of the rapid weight loss. Additionally, the presence, absence, and amount of bowel movements are a reflection of weight cycling during a short period of overfeeding. Additionally, when the fat mass increase started is unknown; thus, further studies are needed to clarify these factors.

Conclusions
TBW is the main component in overfeeding when AEE is maintained at levels seen during normal feeding.

Abbreviations
AEE: activity energy expenditure; BL: baseline; CV: coefficient of variation; EE: energy expenditure; EI: energy intake; FFDS: fat-free dry solid; FM: fat mass; ICC: intraclass correlation coefficient; NEAT: non-exercise activity thermogenesis; OF: overfeeding; PA: physical activity; PFC rate: protein, fat and carbohydrate rate; TBW: total body water; % fat: percent of fat.

Authors' contributions
HS, EY, YY, YH, AK, and YH, conception and design of the study; HS, YJ, EY, YY, MI, and YH, acquisition of data; HS, YJ, AH, EY, YY, MI, HT, and YH, analysis and interpretation of data; HS, YJ, YY, and NE drafting the manuscript; HS, YJ, EY, YH, HT, NE and YH, revising the manuscript; and all of the authors approved the final version of the manuscript.

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第九章

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