1 **Body fat and fatfree mass measured by bioimpedance spectroscopy and dual**

2 **energy x-ray absorptiometry in obese and non-obese adults**

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4 Paula Berstad[1]*, Anna Randby[2,3], Gunn Seim Ekeland[3], Hege Ulveland[4], Torbjørn Omland[2,3], Kari

5 Almendingen[1,5,6]

6

7 [1]*Research Centre, Akershus University Hospital, box 26, 1478 Lørenskog, Norway*

8 [2] *Division of Medicine, Akershus University Hospital, Lørenskog, Norway*

9 [3]*Faculty Division Akershus University Hospital, University of Oslo, Lørenskog, Norway*

10 [4]*Atlantis Medical College, Oslo, Norway*

11 [5]*Faculty of Health, Nutrition and Management, Akershus University College, Lillestrøm, Norway*

12 [6]*Institute of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences,*

13 *Ås, Norway*

14

15 Short title: BIS validation in obese and non-obese adults

16 Key words: **Bioimpedance spectroscopy; Body composition; Dual energy x-ray**

17 **absorptiometry, Obesity; Validation**

18 **Abbreviations:** BIS, Bioimpedance spectroscopy; BMI, body mass index; DXA, dual energy X-ray

19 absorptiometry; ECW, extracellular water; $\Delta_{DXA-BIS}$, difference between the methods DXA and BIS;

20 FM, fat mass; FMkg, FM measured in kg; FM%, FM measured in %; FFM, fatfree mass; FFMkg,

21 FFM measured in kg; FFM%, FFM measured in %; ICW, intracellular water; LoA, limits of

22 agreement; MUAC, Mid-upper arm circumference; WHR, waist-to-hip ratio

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24 *Corresponding author:** Dr Paula Berstad, tel +47 67968775, fax + 47 67968860, email

25 p.m.berstad@medisin.uio.no

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1 **Abstract**

2 The aim of this study was to compare body fat mass (FM) and fatfree mass (FFM) estimates by

3 bioimpedance spectroscopy (BIS) with respective estimates by dual energy X-ray absorptiometry

4 (DXA) in obese and non-obese subjects. Body composition was measured in 93 obese and non-

5 obese men and women by BIS device BodyScout (Fresenius Kabi) and DXA device Lunar iDXA

6 (GE Healthcare). Mean difference between the methods was analyzed by t-tests, and Bland-Altman

7 plots were generated for further examining differences between the methods. Mean difference

8 between the estimates by DXA and BIS ($\Delta_{DXA-BIS}$) (Bland-Altman 95% limits of agreement) were

9 as follows: FM: 4·1 (-2·9 , 11·2) kg, and 4·5 (-2·9 and 11·8) %, FFM: -4·1 (-11·2 and 2·9) kg, and -

10 4·5 (-11·9 and 2·9) %, indicating large inter-individual variation and statistically significant

11 underestimation of FM and overestimation of FFM by BIS as compared to DXA. The

12 underestimation of FMkg and overestimation of FFMkg were more pronounced in men than

13 women, and the underestimation of FM% and overestimation of FFM% were more pronounced in

14 normal weight (body mass index, BMI=20·0-24·9 kg/m^2) than overweight and obese (BMI≥25·0

15 kg/m^2) subjects. BIS may be suitable for classification of a population into groups according to FM

16 and FFM. However, the large inter-individual variation suggests that this BIS device with the

17 proprietary software is in-sufficient for estimation of single individual body FM and FFM.

18

1   Introduction

2   Measurement of body composition is frequently used in the assessment of nutritional status. Low

3   body fatfree mass (FFM) may be a better determinant of malnutrition[1] and mortality[2,3] in patients

4   than low body mass index (BMI). This is becoming more important as prevalence of obesity

5   increases, and malnutrition in different patient groups may be hidden in obesity[4,5]. Consequently,

6   there is need for a simple, valid bedside body composition assessment method.

7   Bioelectrical impedance (BI) is a non-invasive, patient-friendly, portable and relatively

8   inexpensive method for body composition assessment. Bioimpendance technology estimates body

9   water compartments[6,7], based on variation in electrical conductivity between different body tissues.

10   In bioelectrical spectroscopy (BIS), electric current at a spectre of frequencies is led through the

11   body via electrodes placed on skin. Resistivity of extracellular water (ECW) and intracellular water

12   (ICW) compartments against the current varies for different frequencies. High frequent currents

13   penetrate through cell membranes, whereas low frequent currents pass through the body mainly in

14   ECW without penetration into cells. The measured impedance spectral data are fitted into a physical

15   model, giving estimates for ECW and total body water (TBW) volumes. Using these estimates and

16   measured subject weight and height, ICW and further, body FFM and fat mass (FM) can be

17   calculated. BIS is not limited to constant body geometry and relative hydration degree of FFM[6,8,9],

18   which both vary in obesity[8,10]. Therefore, as compared to other BI methods, BIS has higher

19   sensitivity to predict water compartments and FFM in obese subjects or subjects with altered fluid

20   balance[6,9,11].

21   In order to use BIS for FFM estimation in clinical practice on both overweight and normal weight

22   subjects, it has to be validated in a population with a wide spectre of BMIs. Although BIS is

23   supposed to estimate body fluid compartments accurately in normal, healthy subjects[9], the validity

24   may be reduced in obesity[12,13] and in patients in a malnourished state[14]. Dual energy X-ray

25   absorptiometry (DXA) is a widely used reference method for body FFM and FM measurements[9].

26   A recent study on cardiac patients concluded that the BIS device BodyScout (Fresenius Kabi, Bad

Homburg, Germany) only slightly overestimated FFM compared to DXA, but that the large inter-individual variation between the methods indicates that the device does not suit in body composition measurement at individual level[15]. It remains unclear whether the validity of FFM and FM estimates by this BIS device differs between obese and non-obese subjects.

The aim of this study was to examine the validity of BodyScout estimates of FM and FFM of the device, compared to DXA estimates in obese and non-obese adults.

Methods

Subjects

The participants in this study were recruited from The Akershus Sleep Apnea Project, a population-based cross-sectional study in Norway[16]. The aim of the two-phase main study was to assess the prevalence of sleep apnea in Norway. A total of 30.000 subjects aged 30-65 years and living in three different counties in Norway were randomly drawn from the National Population Registry and invited to respond in a questionnaire. A sub-group of 518 responders was further selected for a clinical examination.

We invited 150 randomly drawn participants of the clinical examination to the present sub-study by telephone. Proportion of invited subjects with BMI $\geq$30·0/<30·0 kg/m^2 was 80/20. One-hundred-and-eight subjects gave their consent for participation, while 42 refused to participate. Among those who accepted to participate, 11 subjects were unable to meet up at the measurement appointment. A total of 97 subjects were included in this study, which included anthropometric measurements, a DXA analysis and a BIS test at the Akershus University Hospital. Inclusion criteria for the subjects in the clinical sub-study were not to receive treatment for obstructive sleep apnea. Subjects with significant co-morbid conditions such as cancer, heart failure or severe chronic obstructive pulmonary disease were excluded. Further exclusion criteria in our study were pregnancy, lack of safe contraception in fertile women, and Intra-Cardiac-Device, cochlea implant or large metallic implants operated into the body.

1   This study was conducted according to the guidelines laid down in the Declaration of Helsinki

2   and all procedures were approved by the Regional Ethics Committee, The Norwegian Directorate of

3   Health and Norwegian Social Science Data Services (NSD). Written informed consent was obtained

4   from all subjects. The radiation dose in DXA (0·05-0·15 Gy) was considered very low, less than a

5   standard chest x-ray.

6   *Measurement procedure*

7   The data was collected between May-September 2008. After fasting for a minimum of 8 hours

8   and after avoiding hard physical exercise for 24 hrs, participants were met at the orthopaedic

9   outpatient clinic in the morning. Five subjects reported not being in a fasting state. A trained health

10   specialist (a dietician (HU) or a master of science in physical activity and health (GSE)) performed

11   DXA scanning, the BIS test, and did the blood pressure and anthropometric measurements that

12   included height, weight, and waist, hip and arm circumferences. The participants also filled in a

13   lifestyle questionnaire covering questions on physical activity, weight history, recent weight loss

14   and chronic diseases and disabilities limiting physical activity.

15   *Anthropometric measurements*

16   All anthropometric measurements were conducted while the participant was without shoes and

17   wearing only underwear. Height was measured to the nearest cm, and body weight was measured to

18   the nearest 0·1 kg with a digital scale (Seca, Seca Deutchland, Hamburg, Germany). Mid-upper arm

19   circumference (MUAC) was measured standardized in the non-dominant arm using a scale band at

20   the mid point of upper arm. Waist- and hip circumferences were measured with a scale band,

21   participant standing. Waist circumference was measured between the lowest ribs and *crista iliaca*

22   after an out-breath. Hip circumference was measured at the level of the thickest point of *nates*.

23   *Dual-energy X-ray absorptiometry*

24   The DXA instrument used in this study was Lunar iDXA (GE Healthcare, Madison, WI, USA).

25   Whole-body scans were performed according to manufacturer's instructions, and body fat, lean

26   tissue mass (LTM) and bone mineral content (BMC) were analysed (software enCore version

1   11.10). The daily quality control procedure of the device was conducted automatically, and included

2   a six-point calibration of three different bone-simulating chambers of known BMC and three

3   different soft tissue-simulating chambers of known fat percent. The program automatically

4   determined total body measurement mode according to participant thickness (thick, >25 cm;

5   standard, 13-25 cm; and thin, <13 cm)[17]. DXA estimate of FFM was calculated as a sum of LTM

6   and BMC estimates. Precision of LTM and BMC estimates of the Lunar iDXA device have been

7   found satisfactory by others (coefficient of variation 0·5% and 0·6%, respectively)[18].

8   Nineteen subjects were too large for the DXA instrument. These were scanned one-sided, and the

9   instrument estimated measurement results for the whole body. Agreement in fat and lean mass

10   estimates between whole-body DXA scan and estimates assessed by half-body DXA scan in obese

11   subjects has been reported[19].

12   *Bioelectric impedance spectroscopy*

13   We conducted the BIS measurement after the participant had taken off all electricity leading items

14   such as belts, watches and jewellery, and rested in a supine position for 5 min. During the

15   measurement, the participant was lying supine with four tactile electrodes placed on the skin, two

16   on the dorsal surface of the dominant hand/wrist and two on the parallel foot/ankle according to the

17   manufacturer's instructions. The BIS analyser used was BodyScout (Fresenius Kabi, Bad Homburg,

18   Germany). The BodyScout device covers 50 frequencies in a range of 5 kHz to 1 MHz. The

19   proprietary software extrapolates ECW and ICW using the Hanai equation[20], as described by

20   Moissl et al.[21]. The BodyScout estimates of FM and FFM are not sex specific, and do not expect a

21   fixed ECW/ICW ratio. The body composition model used in the BodyScout software is based on a

22   principal that whole-body mass (M_{WB}) is a sum of three compartments: normally hydrated adipose

23   tissue mass (M_{NH_AT}), normally hydrated lean tissue mass (M_{NH_LT}), and excess fluid (ExF) mass

24   (M_{ExF}), as described by Chamney et al.[22,23]:

25

26   $$M_{WB} = M_{NH\_AT} + M_{NH\_LT} + M_{ExF} \tag{1}$$

1

2   The total water (TW), ICW and ECW components of M_{NH_LT} and M_{NH_AT} are defined by equations

3   2-7.

4

5    $$H_{TW\_NH\_LT} = M_{TW\_NH\_LT}/M_{NH\_LT} \qquad (2)$$

6    $$H_{ECW\_LT} = M_{ECW\_NH\_LT}/M_{NH\_LT} \qquad (3)$$

7    $$H_{ICW\_NH\_LT} = M_{ICW\_NH\_LT}/M_{NH\_LT} \qquad (4)$$

8    $$H_{TW\_NH\_AT} = M_{TW\_NH\_AT}/M_{NH\_AT} \qquad (5)$$

9    $$H_{ECW\_NH\_AT} = M_{ECW\_NH\_AT}/M_{NH\_AT} \qquad (6)$$

10   $$H_{ICW\_NH\_AT} = M_{ICW\_NH\_AT}/M_{NH\_AT} \qquad (7)$$

11

12   where $H_{TW\_NH\_LT}$, H_{ECW_LT}, $H_{ICW\_NH\_LT}$, $H_{TW\_NH\_AT}$, $H_{ECW\_NH\_AT}$ and $H_{ICW\_NH\_AT}$ are fractional mass

13   of TW, ECW and ICW in normally hydrated lean tissue, respectively, and $H_{TW\_NH\_AT}$, $H_{ECW\_NH\_AT}$

14   and $H_{ICW\_NH\_AT}$ are fractional mass of TW, ECW and ICW in normally hydrated adipose tissue,

15   respectively. In the BodyScout software, total body FM in kg (FMkg) is related to M_{NH_AT} with

16   equation 8.

17

18   $$FMkg = M_{NH\_AT} \text{ x } (1 - H_{TW\_NH\_AT} - K_{AR}) \qquad (8)$$

19

20   where K_{AR} is the ratio of residual adipose components (solids, mainly protein and mineral) to

21   M_{NH_AT} with a value of typically 0·05[24,25]. FFMkg is calculated by the software by extracting

22   FMkg from body weight.

23   BIS estimate of FFM (FFMkg$_{TBW/0·73}$) was calculated also directly from TBW according to

24   equation 9, based on the assumption of a 73% hydration of the fatfree mass[26].

25

26   $$FFMkg_{TBW/0·73} = TBW/0·73 \qquad (9)$$

1 Further, $FMkg_{TBW/0.73}$ was calculated as $FFMkg_{TBW/0.73}$ extracted from body weight.

2 *Lifestyle questionnaire*

3 In the lifestyle questionnaire, the participants were asked to report how often during the last year

4 they were involved in non-vigorous and vigorous physical activity, choosing between 4 levels for

5 both: Never, less than 1 h/week, 1-2 h/week and 3 h/week or more. They were also asked to report

6 their habitual activity level with four choices: sedate, light activity 4 h/week or more, condition

7 training 4 h/week or more and regular hard physical training. The participants were asked to

8 describe their smoking status by choosing between non-smoker or current smoker, and to give the

9 number of cigarettes smoked daily, years since smoking start and cessation, and how many years

10 totally smoked. The participants were asked to assess their body weight at ages 18, 30, 40, 50 and

11 60y, 6 and 12 months prior to the study date, and the lowest and highest weight since 18y age. The

12 questionnaire also included a question on whether the participant had one or more chronic diseases

13 or disabilities limiting physical activity, and if answering "Yes", the participant was asked to write

14 down the disease/disability.

15 *Pilot study*

16 We conducted a pilot study on the anthropometric, BIS and DXA measurements in 20 hospital

17 employees and other voluntary subjects recruited by the project team in order to study variability in

18 measurements between the two project team members who conducted the measurements (HU and

19 GSE). There was no statistically significant difference in the measurements of arm, waist and hip

20 circumference or BIS and DXA measurements between the measurers (One-Sample T-test, all

21 $P \geq 0.21$). There was a statistically significant slight difference between the measurers in height and

22 weight measurements (mean differences 0·1 cm and 0·1 kg), but this difference was without clinical

23 significance.

24 *Statistical analysis*

25 Power calculation. Assuming that the participants have an average weight of 90 kilos and 40% fat,

26 gave estimated total fat mass of around 36 ± 3 kg. Given a α of 5% and statistical power (1-β) of

1   80%, the minimum number of obese subjects to be included in each group, to detect a difference of

2   2 kg fat mass, was then $n = 2 \times (3/2)^2 \times 7 \cdot 9 = 35$ per group. We also invited 20 healthy and normal

3   weight persons, to make sure to have a control group with optimal measurement conditions and

4   results.

5   Data analysis. There is a considerable difference between the BIS and DXA technologies in

6   estimating FMkg and FFMkg. In DXA, FM is directly estimated in kg, and FFMkg can be

7   calculated as the sum of LTM and BMC estimates in kg. The percentage estimates of FM and FFM

8   are calculated using the sum of all body mass compartments in kg as body weight. In BIS, body

9   weight obtained from an independent measurement (a digital scale in our study) is inserted into the

10   software prior to the measurements. FMkg and FFMkg estimates are calculated using the

11   percentage estimates of these and the given body weight in kg. The mean body weight measured by

12   the digital scale in our study was 0·5 kg (SD 0·8 kg) higher than the mean DXA estimate of body

13   weight. This did, however, not significantly differ between obese and other subjects, overweight

14   and normal weight subjects, or half- versus whole- DXA-scanned subjects (data not shown).

15   Consequently, the FMkg and FFMkg estimates of DXA were converted to values the sum of which

16   totalled body weight by the digital scale. We calculated physical activity score as a sum of scores

17   from non-vigorous and vigorous activity, giving score 0-3 for the four choices of non-vigorous

18   activity and double score for the respective choices of vigorous activity. This resulted in total

19   physical activity score range from 0 to 9. We further categorized the total physical activity score

20   into low (≤3), middle (4-6) and high (7-9) activity groups. We gave score 0-2 for the habitual

21   physical activity choices, combining the two choices with highest activity. Smoking status was

22   reported as never-, current and ex-smoker. Weight change during the preceding 12 months was

23   calculated using the self-reported weight for 12 months earlier and the measured weight. Diseases

24   and disabilities limiting physical activity were categorized according to type of disease/disability.

25   Descriptive statistics are reported as distributions and as mean values with SD. Bland-Altman

26   plots[27], a well-known tool for comparing a new and a referent method, were used to present

1 differences between FM and FFM estimates by DXA and BIS. The limits of agreement (LoA)

2 between the two methods were defined as mean difference $\pm 1\cdot96$ SD[27]. We tested mean difference

3 between the methods ($\Delta_{DXA-BIS}$) with paired-samples t-test, and used independent-samples t-test (if

4 two subgroups) or one-way ANOVA (if three subgroups) to analyse differences in $\Delta_{DXA-BIS}$ between

5 subgroups. We considered two-tailed $P<0\cdot05$ as statistically significant. We conducted the

6 statistical analysis using software SPSS 17.0.1 for Windows (SPSS Inc., Chicago, IL, USA).

7 **Results**

8   Subject characteristics are as described in Table 1. A total of 93 participants (61% males) with

9 successful measurements were included in the study, with mean age of 51 years. The majority of the

10 participants (59 subjects, 63%) were obese (BMI$\geq30\cdot0$ kg/m^2), while 22 subjects (24%) were

11 overweight (BMI=$25\cdot0$-$29\cdot9$ kg/m^2) and 12 subjects (13%) had a normal BMI ($20\cdot0$-$24\cdot9$ kg/m^2).

12 Some participants had changed weight between the clinical examination of the main study and the

13 present study measurement date, therefore the intended proportion of subjects with BMI

14 $\geq30\cdot0/<30\cdot0$ kg/m^2 (80/20) was some changed. Forty-one percent of the participants reported

15 chronic diseases or disabilities limiting physical activity.

16   FFM as kg and proportion (FFMkg and FFM%, respectively) were significantly higher in men

17 than women, whereas FM as proportion (FM%) was significantly higher in women, estimated with

18 both DXA and BIS (all $P<0\cdot001$). There was no significant difference in FM as kg (FMkg) between

19 the sexes (Table 1).

20 *FM comparison*

21   BIS significantly underestimated mean FMkg and FM% as compared to DXA (Table 2 and Fig.

22 1a and b). Mean differences of the estimates between the methods ($\Delta_{DXA-BIS}$) and Bland-Altman

23 95% LoA:s were $4\cdot1$ ($-2\cdot9$ and $11\cdot2$) kg and $4\cdot5$ ($-2\cdot9$ and $11\cdot8$) % for FMkg and FM%, respectively

24 (Table 2 and Fig. 1a and b). The estimates by the two methods were highly correlated (Table 2).

25 There was a statistically significant inverse correlation between the difference and mean values of

26 FM ($r_{Pearson}$=$-0\cdot27$ and $-0\cdot54$, for FMkg and FM%, respectively, $P<0\cdot01$ for both, Fig. 1a and b).

1   Underestimation of FMkg was significantly more pronounced in men than in women ($\Delta_{\text{DXA-BIS}}$ (SD)

2   4·8 (3·8) vs. 3·1 (3·0) kg, P=0·02) (Table 3). Underestimation of FM% was significantly more

3   pronounced in normal weight subjects than overweight and obese subjects ($\Delta_{\text{DXA-BIS}}$ (SD) 6·9 (2·7)

4   vs. 4·1, (3·8) %, P=0.005) (Table 4). Estimation difference for FM% between the two methods was

5   significantly inversely correlated with the FM% estimate by DXA (r_{Pearson}=-0·29, P=0·005) (data not

6   shown). The BIS estimates calculated as 73% hydration of FFM ($\text{FMkg}_{\text{TBW/0·73}}$ and $\text{FM\%}_{\text{TBW/0·73}}$)

7   were significantly higher than the respective BIS estimates by the proprietary software.

8   Consequently, the underestimation of these FM values was significantly smaller than for these

9   estimates given by the BIS software. However, the method differences for $\text{FMkg}_{\text{TBW/0·73}}$ and

10     $\text{FM\%}_{\text{TBW/0·73}}$ ($\Delta_{\text{DXA-BIS(TBW/0·73)}}$) were statistically significant (Table 2) and all results for $\text{FM}_{\text{TBW/0·73}}$

11   estimates close to identical with FM estimates by BIS software (data not shown).

12   There was neither statistically significant difference in the underestimation of FMkg or FM% by

13   BIS between obese (BMI$\geq$30·0 kg/m^2) and other subjects, nor between very obese (BMI$\geq$34·0

14   kg/m^2, 18 subjects) and other subjects, or between subjects with half- and whole-body DXA scan

15   (data not shown). There was no difference in the underestimation through age groups, physical

16   activity scores or recent weight change groups (data not shown).

17   When grouping subjects into FMkg and FM% quartiles by BIS and DXA estimates, 74 and 73%

18   of the subjects, respectively, were classified into the same quartile by the two methods, whereas 26

19   and 25%, respectively were classified into same ± 1 quartile (Table 5).

20   *FFM comparison*

21   BIS significantly overestimated mean FFMkg and FFM% as compared to DXA (Table 2 and Fig.

22   1c and 1d). $\Delta_{\text{DXA-BIS}}$ and Bland-Altman LoA:s were -4·1 (-11·2 and 2·9) kg and -4·5 (-11·9 and 2·9)

23   % for FFMkg and FFM%, respectively (Table 2 and Fig. 1c and d). The estimates by the two

24   methods were highly correlated (Table 2). There was a statistically significant inverse correlation

25   between the difference and mean values of FFM (r_{Pearson}=-0·36 and -0·53, for FFMkg and FFM%,

26   respectively P<0·01 for both, Fig. 1c and d). Overestimation of the absolute FFMkg was

1   significantly more pronounced in men than in women ($\Delta_{DXA-BIS}$ (SD) -4·8 (3·8) vs. -3·1 (3·0) kg,

2   *P*=0·03) (Table 3). Additionally, overestimation of FFM% was significantly more pronounced in

3   normal weight subjects than overweight and obese subjects ($\Delta_{DXA-BIS}$ (SD) -6·9 (2·8) vs. -4·1 (3·8)

4   %, *P*=0·006) (Table 4). Estimation difference for FFM% between the two methods was

5   significantly inversely correlated with the FFM% estimate by DXA ($r_{Pearson}$=-0·33, *P*=0·001) (data

6   not shown). The BIS estimates calculated as 73% hydration of FFM (FFMkg$_{TBW/0·73}$ and

7   FFM%$_{TBW/0·73}$) were significantly lower than the respective BIS estimates by the proprietary

8   software. Consequently, the overestimation of these FFM values was significantly smaller than for

9   these estimates given by the BIS software. However, the method differences for FFMkg$_{TBW/0·73}$ and

10   FFM%$_{TBW/0·73}$ ($\Delta_{DXA-BIS(TBW/0·73)}$) were statistically significant (Table 2) and all results for

11   FFM$_{TBW/0·73}$ estimates close to identical with FFM estimates by BIS software (data not shown).

12   There was neither statistically significant difference in the overestimation of FFMkg or FFM% by

13   BIS between obese (BMI≥30·0 kg/m^2) and other subjects, nor between very obese (BMI≥34·0

14   kg/m^2, 18 subjects) and other subjects, or between subjects with half- and whole-body DXA scan

15   (data not shown). There was no difference in the overestimation through age groups, physical

16   activity scores or recent weight change groups (data not shown).

17   When grouping subjects into FFMkg and FFM% quartiles by BIS and DXA estimates, 74 and

18   71% of the subjects, respectively, were classified into the same quartile by the two methods,

19   whereas 26 and 26%, respectively were classified into same ± 1 quartile (Table 5).

20   **Discussion**

21   In this validation study, we observed large inter-individual variation in FM and FFM estimates

22   between BIS and DXA. We observed a significant underestimation of FM and overestimation of

23   FFM by BIS as compared to DXA. The underestimation of FMkg and the overestimation of FFMkg

24   were higher in men than to women. The underestimation of FM% and the overestimation of FFM%

25   were both higher at normal compared to high BMI. Further, underestimation of FM% was more

26   pronounced at low FM% and overestimation of FFM% was more pronounced at high FFM%. The

12

FFM and FM estimates calculated from TBW by BIS were closer to these estimates from DXA. The large inter-individual variation and heterogeneity in validity between subgroups indicate that BIS is not a suitable method for single body fat or fatfree mass assessment at an individual level. The present BIS device BodyScout with the proprietary software was initially designed to estimate body liquid compartments. Our study demonstrates that it may not be equally suitable for estimation of body fat and fatfree mass.

In our population with obese and non-obese subjects, using the present BIS device, the BIS estimates of FM and FFM will be significantly better if these estimates are calculated directly from TBW by BIS, expecting 73% hydration of FFM. Such observation has been done earlier by Ellegård et al.[14]. Earlier comparisons in different population groups have, in line with our results, found large inter-individual variation in mean difference between BIS and DXA, accompanied by overestimation of FFM[15,28,29] and underestimation of FM[29] by BIS. Underestimation of FFM by BIS has also been reported, but in incurable cancer patients[14]. In contrast to our study, the overestimation of FFM by BIS was in most previous studies in adults not related to BMI, other anthropometric variables, sex[28,29] or magnitude of FFM and FM estimates[29]. In one study, overestimation of FFM by BIS compared to DXA was related to overweight[13]. In children, BIS has been found to overestimate FM in lean, and underestimate FM in overweight subjects[30]. Higher validity of FFM% and FM% estimates in overweight than normal weight subjects is to our knowledge reported only by our study. The number of normal weight subjects in the present study (n 12) probably is too low to draw conclusions on this. However, the significant correlations between the method difference ($\Delta FM\%_{DXA-BIS}$ and $\Delta FFM\%_{DXA-BIS}$) and the FM% and FFM% values, respectively, indicate an inverse dose-response association between validity of these estimates and adiposity of the subject.

BIS validation studies against other than DXA have compared BIS estimates of ECW with dilution methods and ICW with total body potassium. These have found reduced BIS validity in disease state or obesity[12,21]. A suggested explanation for reduced validity of BIS measurements in

1 obesity is the sensitivity of BIS to detect imbalance in body water compartments. BIS estimates of

2 ECW and ICW are altered by changes in these, as found by Moissl in patients with renal failure and

3 healthy subjects[21]. The ECW/ICW ratio is greater in obese due to increased amount of ECW and

4 TBW[10] as compared to non-obese subjects, leading to overestimation of FFM and underestimation

5 of FM in obesity. Although BIS is sensitive to body water changes in normal, healthy subjects[9],

6 accuracy of BIS estimates of body water decreases in obesity[12]. The opposite, a larger

7 measurement error by BIS in normal weight subjects in our study, cannot be explained by this

8 reduced BIS sensitivity in an abnormal body fluid balance. However, we observed larger

9 measurement error by BIS in normal weight subjects only in the percentage proportions of FFM and

10 FM. The reduced validity of BIS in altered fluid balance has been associated rather with the

11 absolute estimates of ICW and ECW in litre[12,21].

12 Another possible explanation for the large inter-individual variability between BIS and DXA may

13 be a reduced validity of DXA measurements in obese subjects. Although DXA has been found to be

14 an accurate method for body fat and lean tissue measurements[31], underestimation of FFM in

15 women and reduced validity in FFM estimates by truncal adiposity[32] and increasing body size[33]

16 have been reported. DXA has been suspected to underestimate FM in severely obese subjects as a

17 result of increased photon absorption[34]. If the DXA measurements in our study were similarly

18 biased, the observed underestimation of FM by BIS in obese subjects may have been smaller than

19 the real underestimation in these subjects. In that case, the difference in BIS measurement error

20 between normal weight and overweight subjects would in reality be smaller than observed. We did

21 not observe differential method difference between the subjects scanned one-sided by DXA (*n* 19)

22 and others. We can therefore not suspect a reduced precision of the DXA device in estimation of

23 total body FM and FFM from the one-sided DXA scan in this study.

24 It has been suggested that BIS suits for assessment of body composition in groups or individual

25 body composition changes rather than determination of single individual body composition[35]. BIS

26 estimates of individual TBW measurements have not been considered valid in a population with

1   wide range of BMIs[36], but BIS has been reported to be a reproducible method within an

2   individual[37,38] and track intra-individual changes in TBW accurately, regardless of BMI[36,38].

3   Estimating changes in body composition may be the most applicable use of BIS in clinical practice,

4   although a recent study suggested that BIS is inappropriate also for this purpose because of the large

5   inter-individual variability[15]. We found that BIS classifies the population relatively accurately into

6   quartiles according to FM and FFM. This may be satisfactory for epidemiological studies. Body

7   composition assessment is, however, mainly used in clinical studies and accurate individual

8   measurements are necessary. Hence, correct ranging into groups is not sufficient for validity of the

9   device.

10   Careful participant recruitment and standardized examination procedures are strengths of this

11   study. The pilot study conducted before the data collection showed agreement in measurements

12   between the two measurers. Corrections were made in height and weight measurement procedures

13   in order to minimize the slight measurer difference. The small number of normal weight subjects is

14   a limitation of this study. Use of validated regression equations have been warranted as a condition

15   of clinical bioimpedance technology use in subjects with abnormal body shape[6]. We did not for the

16   purpose of this study develop new predicted equations based on BodyScout estimates of body fluid

17   compartments, resistance and BMI, as our aim was to validate the BodyScout device "as is". The

18   number of normal weight subjects in our sample would probably have been too low to develop

19   equations useful for clinical practice.

20   We conclude that we found a large inter-individual agreement in FM and FFM estimates between

21   the present BIS device and DXA. We also observed that this BIS device underestimates FM and

22   overestimates FFM. In agreement with a recent validation study[15], we conclude that this BIS

23   device using the proprietary software has too low accuracy to be used in estimation of body fat and

24   fatfree mass at individual level. The absolute BIS estimates in kg may be less valid in men than

25   women, and may be less comparable with DXA estimates than the proportional estimates. In

26   epidemiological studies, a population can be satisfactorily classified into groups by the FFM and

1 FM estimates. Finally, we suggest that future validation studies on BIS among obese subjects

2 include golden standard methods for estimation of body fluids.

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1 **Tables**

2 **Table 1.** Characteristics of study participants (*n* 93)

Variable	All (*n* 93)		Men (*n* 57)		Women (*n* 36)	
	Mean	SD	Mean	SD	Mean	SD
Age (years)	51·0	11·5	51·7	10·5	49·7	13·0
Weight (kg)	94·6	16·5	100·4	14·8	85·4	15·0
Body mass index (kg/m^2)	30·9	4·5	31·2	4·5	30·5	4·6
Waist-hip ratio	0·96	0·10	1·01	0·07	0·88	0·07
Arm circumference (cm)	33·2	3·2	33·9	2·8	32·0	3·4
DXA-derived data						
FMkg (kg)	35·2	10·2	34·2	9·8	36·7	10·7
FM% (%)	36·9	7·2	33·5	5·5	42·3	6·2
FFMkg (kg)	59·4	10·7	66·1	6·7	48·6	6·0
FFM% (%)	63·1	7·2	66·5	5·5	57·7	6·2
BIS-derived data						
ECW/ICW ratio	0·80	0·08	0·78	0·07	0·83	0·08
FMkg (kg)	31·1	11·2	29·5	10·9	33·6	11·2
FM% (%)	32·5	9·2	28·6	7·9	38·6	7·7
FFMkg (kg)	63·5	12·0	70·9	7·9	51·8	7·0
FFM% (%)	67·6	9·2	71·4	7·9	61·5	7·7
Re (ohm)	549	77	511	51	611	72
Ri (ohm)	1122	255	992	161	1329	240
Resistence at 50 kHz (ohm)	449	72	410	44	510	64
Reactance at 50 kHz (ohm)	51·2	7·3	49·6	6·6	53·7	7·8
Phase angle (°)	6·6	0·8	6·9	0·7	6·0	0·7
	n	%	n	%	n	%
Abdominal obesity waist circum. >102cm (males), >88cm (females)	65	69·9	39	68·4	26	72·2
High waist-hip ratio >1.00 (males), >0.85 (females)	57	61·3	35	61·4	22	61·1
Weight reduction ≥10% during the last 12 months	6	6·5	3	5·3	3	8·3
Weight reduction ≥5% during the last 12 months	18	19·4	9	15·8	9	25·0
Smoking status						
Never smoker	40	43·0	27	47·4	13	36·1
Ex-smoker	36	38·7	25	43·9	11	30·6
Daily smoker	17	18·3	5	8·8	12	33·3
Chronic diseases/disabilities limiting physical activity						
Combined	15	16·1	9	15·8	6	16·7
Knees/Hips	9	9·7	4	7·0	5	13·9
Cardiac	6	6·5	5	8·8	1	2·8
Back/prolaps	3	3·2	3	5·3	-	
Other*	5	5·5	-		5	13·9

3 BIS, Bioelectrical impedance spectroscopy; DXA, Dual-energy X-ray absorptiometry; ECW, extracellular water; FM,
4 fat mass; FFM, fatfree mass; ICW, intracellular water; Re, resistance of extracellular volume; Ri, resistance of
5 intracellular volume
6 *Rheumatic, pulmonary or psychiatric problems, fibromyalgi or other.

1   **Table 2.** Comparison of FM and FFM estimates by DXA, and BIS by the proprietary software (BIS$_{software}$) and by direct calculation from TBW by BIS
2   All subjects (*n* 93)

	DXA		BIS$_{software}$†		Correlation (DXA-BIS$_{software}$) $r_{Pearson}$	Difference between the methods, $\Delta_{DXA-BIS(software)}$		BIS$_{TBW/0.73}$‡		Correlation (DXA-BIS$_{TBW/0.73}$) $r_{Pearson}$	Difference between the methods, $\Delta_{DXA-BIS(TBW/0.73)}$	
	Mean	SD	Mean	SD		Mean	SD	Mean	SD		Mean	SD
FMkg	35·2	10·2	31·1	11·2	0·95	4·1***	3·6	31·7	10·5	0·96	3·5***	3·1
FM %	36·9	7·2	32·5	9·2	0·92	4·5***	3·8	33·2	8·3	0·93	3·8***	3·1
FFMkg	59·4	10·7	63·5	12·0	0·96	-4·1***	3·6	62·9	11·6	0·97	-3·5***	3·1
FFM%	63·1	7·2	67·6	9·2	0·92	-4·5***	3·8	66·8	8·3	0·93	-3·8***	3·1

3   BIS, Bioelectrical impedance spectroscopy; DXA, Dual-energy X-ray absorptiometry; FM, fat mass; FFM, fatfree mass; TBW, total body water
4   Mean difference between the DXA and BIS methods statistically significant: ***$P<0.001$.
5   †FM and FFM estimated by BIS software as described by Chamney et al.[22]
6   ‡FM and FFM estimated by direct calculation from TBW by BIS, expecting 73% hydration of FFM[26]
7
8
9
10   **Table 3.** Difference between the methods ($\Delta_{DEXA-BIS}$) in FM and FFM estimates in men and women
11   Mean differences and standard deviations

	Men (*n* 57)		Women (*n* 36)	
	Mean	SD	Mean	SD
$\Delta FMkg_{DXA-BIS}$	4·8	3·8	3·1*	3·0
$\Delta FM\%_{DXA-BIS}$	4·9	3·9	3·8	3·5
$\Delta FFMkg_{DXA-BIS}$	-4·8	3·8	-3·1*	3·0
$\Delta FFM\%_{DXA-BIS}$	-4·9	3·9	-3·8	3·5

12   BIS, Bioelectrical impedance spectroscopy; DXA, Dual-energy X-ray absorptiometry; FM, fat mass; FFM, fatfree mass
13   Mean difference statistically significant from men: *$P<0.05$.
14

1

2 **Table 4.** Difference between the methods ($\Delta_{DEXA-BIS}$) in FM and FFM estimates in normal weight (BMI<25 kg/m^2) and overweight (BMI≥25 kg/m^2)

3 subjects

4 Mean differences and standard deviations

	Normal weight subjects (BMI<25 kg/m^2) (*n* 12)		Overweight subjects (BMI≥25 kg/m^2) (*n* 81)	
	Mean	SD	Mean	SD
$\Delta FMkg_{DXA-BIS}$	5·0	2·3	4·0	3·7
$\Delta FM\%_{DXA-BIS}$	6·9	2·7	4·1**	3·8
$\Delta FFMkg_{DXA-BIS}$	-5·0	2·3	-4·0	3·7
$\Delta FFM\%_{DXA-BIS}$	-6·9	2·8	-4·1**	3·8

5 BIS, Bioelectrical impedance spectroscopy; DXA, Dual-energy X-ray absorptiometry; FM, fat mass; FFM, fatfree mass

6 Mean difference statistically significant from normal weight subjects: ** P<0·01.

7

8

9

10 **Table 5.** Joint classification of subjects by quartiles of FM and FFM estimated by DXA and BIS (*n* 93)

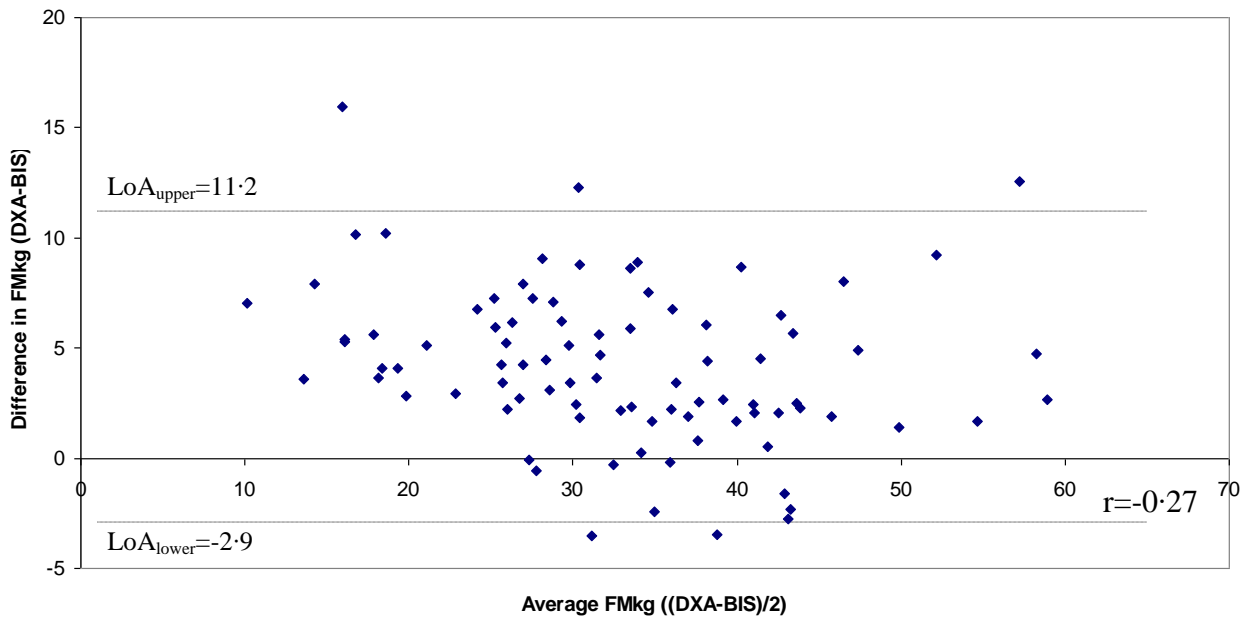|	Classified into same quartile	Classified into same ± 1 quartile	Classified into opposite quartiles
	%		
FMkg	74	26	0
FM%	73	25	2
FFMkg	74	26	0
FFM%	71	26	3

11 BIS, Bioelectrical impedance spectroscopy; DXA, Dual-energy X-ray absorptiometry; FM, fat mass; FFM, fatfree mass
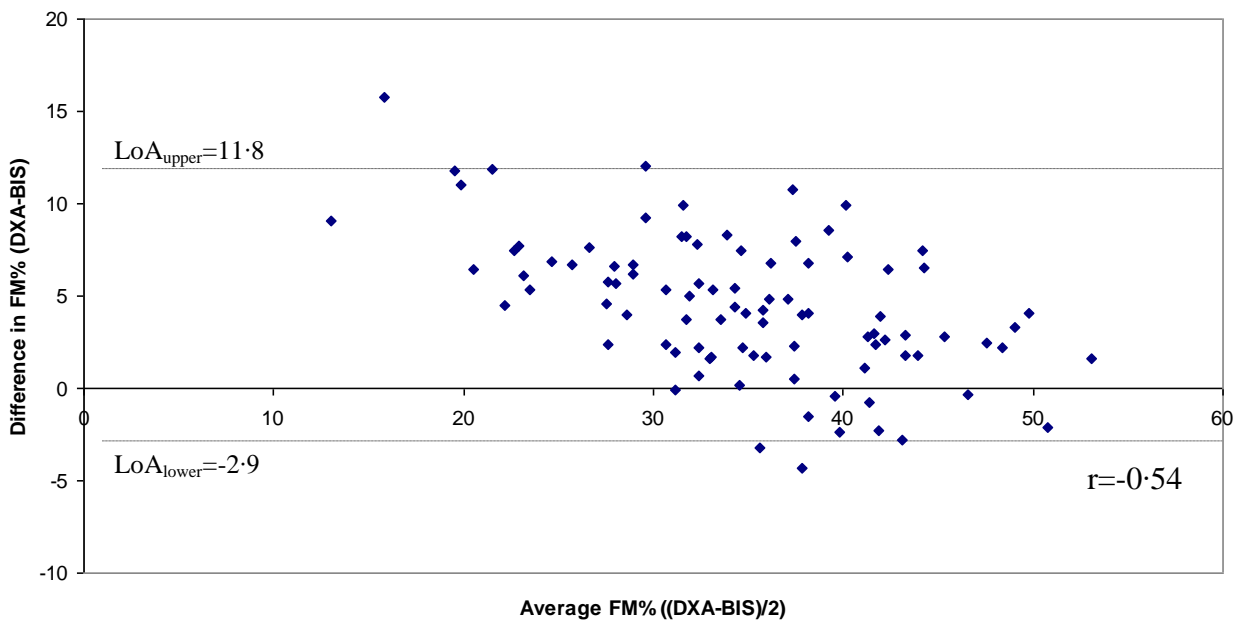
1   Figure legend.
2
3   **Figure 1.** Bland-Altman plots of comparisons of bioelectrical impedance spectroscopy (BIS) with
4   dual-energy X-ray absorptiometry (DXA) estimates for a) body fat mass in kg (FMkg), b) body fat
5   mass as percentage (FM%), c) body fatfree mass in kg (FFMkg), and d) body fatfree mass as
6   percentage (FFM%) with limits of agreement (LoA) (dashed lines) and Pearson correlation
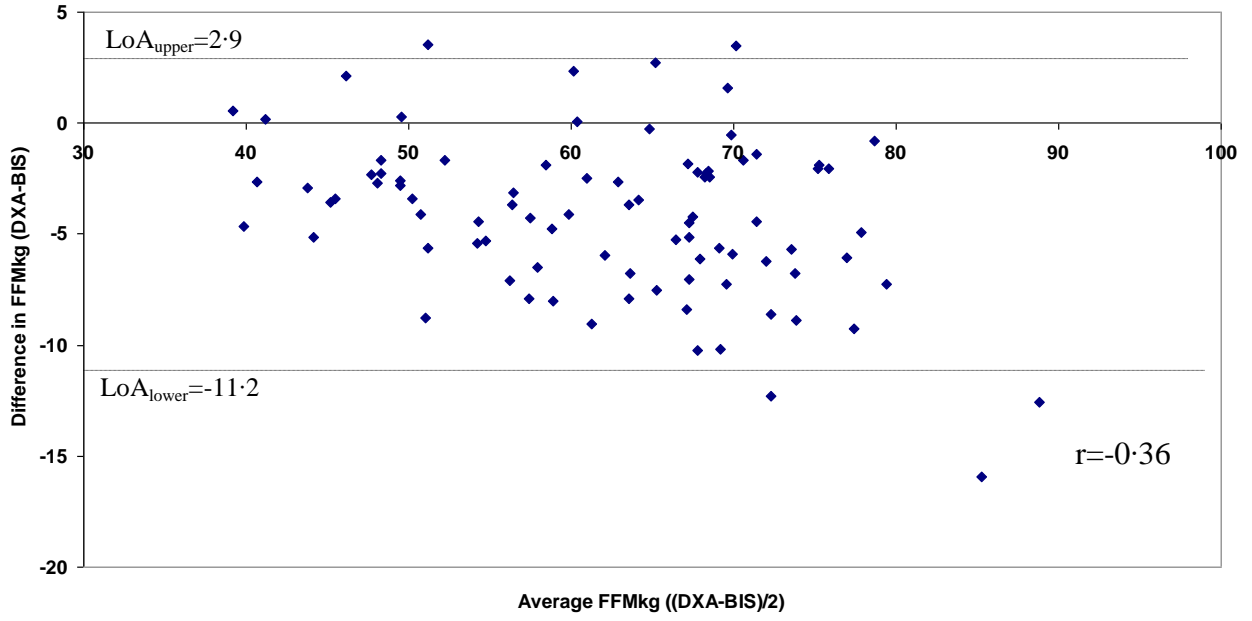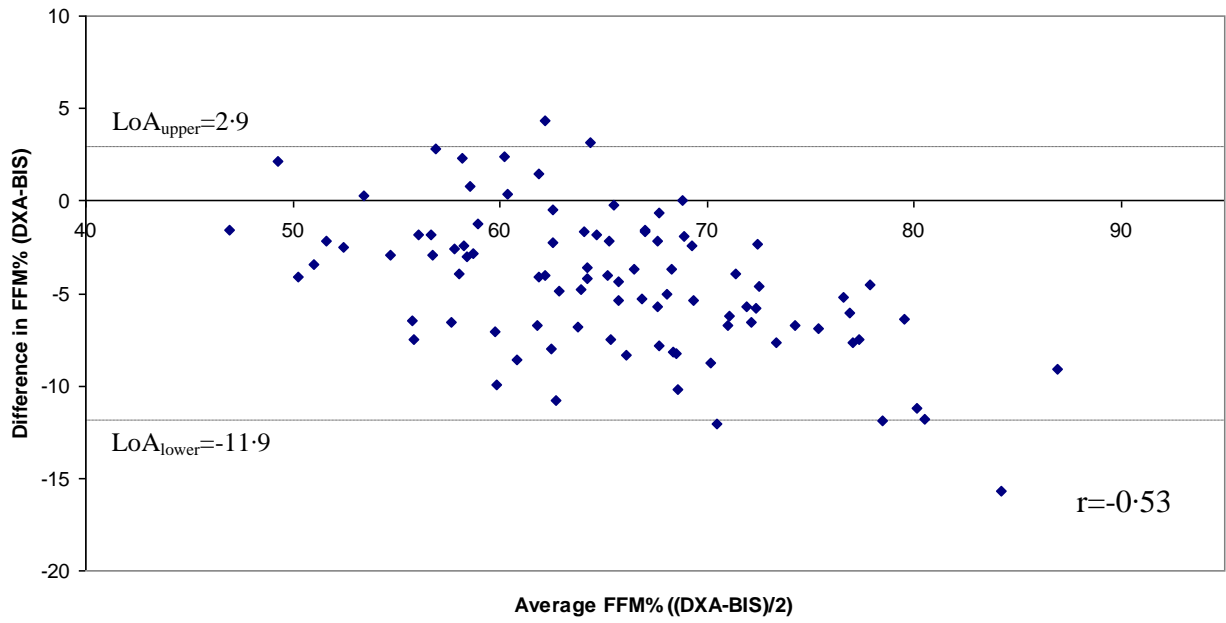7   coefficient (r) (*n* 93)
8

20

15

LoA$_{upper}$=11·2

10

5

0

10   20   30   40   50   60   r=-0·27   70

-5

LoA$_{lower}$=-2·9

Difference in FMkg (DXA-BIS)

Average FMkg ((DXA-BIS)/2)

1
2   1a.
3

20

15

LoA$_{upper}$=11·8

10

5

0

10   20   30   40   50   60

LoA$_{lower}$=-2·9

-5   r=-0·54

-10

Difference in FM% (DXA-BIS)

Average FM% ((DXA-BIS)/2)

4
5   1b.
6
7

1

Average FFMkg ((DXA-BIS)/2)

2
3   1c.
4
5
6

Average FFM% ((DXA-BIS)/2)

7
8   1d.
9
10