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Whole Body Composition by Hologic QDR 4500/A DXA: System Reliability versus User Accuracy and Precision

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1. Introduction

Accurate and precise measurement of human tissue composition is both important and imperative in individual health assessment. Although body composition (BC) data acquisition and analysis are both popular and important, selecting an appropriate method or technique for accurate and/or precise assessment of individuals and/or groups remains a challenging task within various sectors of public health. Since 1950s and 1960s, with the pioneer work of Keys & Brozek (1953), Forbes et al. (1956), Siri (1956), Brozek et al. (1963), Behnke (1963), Durnin & Rahaman (1967), BC almost became a scientific discipline profiling itself with the development of many methods, techniques, and equipments. Popular approaches have been criticized over the years because they are subject to measurement errors and/or violation of basic assumptions underlying their use such as hydrodensitometry (Clarys et al., 2010c; Clasey et al., 1999; Elowsson et al., 1998; Heyward, 1996; Johansson et al., 1993; Prior et al., 1997) or anthropometry, e.g., skinfolds (Beddoe, 1998; Clarys et al., 1987, 2005, 2010a; Martin et al., 1985, 1992; Scafoglieri et al., 2010a) and the universally accepted new method of choice, the dual energy X-ray absorptiometry (DXA) (Bolotin, 1998, 2007; Bolotin & Sievanen, 2001; Bolotin et al., 2001; Clarys et al., 2010b; Provyn et al., 2008; Scafoglieri et al., 2010c).

1.1 New gold standard for BC data acquisition?

After reviewing the literature of DXA application, one obtains a controversial impression of this new method. On the one hand, we find an important number of validation and application studies that support the DXA technique as convenient, as the criterion for fat percentage, lean body mass (LBM), and bone mineral content (BMC) (Clasey et al., 1999; Haarbo et al., 1991; Johansson et al., 1993; Prior et al., 1997; Pritchard et al., 1993). A number of authors as mentioned in Provyn *et al.* (2008) suggest DXA as the gold standard for validation of other techniques essential for the measurement of BC (Eston et al., 2005; Poortmans et al., 2005; Salamone et al., 2000). On the other hand it needs reminded that DXA, likewise hydrodensitometry, anthropometry, bioelectrical impedance, air, gas, and water displacement methods, is an indirect *in vivo* technique for measuring BC. Validation or even cross-validation in between indirect methods cannot guarantee both accuracy and

reality precision. Perfect correlations and low coefficients of variation allow for good predictions and assumptions only (Bolotin & Sievanen, 2001; Clarys et al., 2010b; Provyn et al., 2008).

1.2 Data acquisition quality issues

Possibly the greatest problems with accuracy/precision in DXA are found with fat and lean tissue estimates (Prentice, 1995), with its projected areal bone density (Bolotin, 2007; Bolotin et al., 2001; Clarys et al., 2008, 2010b; Provyn et al., 2008; Scafoglieri et al., 2010c) and with the basic confusion between overall BC terminology, e.g., fat, adipose tissue (AT), fat-free mass (FFM), LBM, lean and AT free mass (ATFM), bone mineral density (BMD), surface and volume density, BMC, ash weight, actual mineral content, and BMC, with or without soft tissue (ST) covering (Clarys et al., 2010b; Martin et al., 1985; Provyn et al., 2008; Scafoglieri et al., 2010b; Wadden & Didie, 2003). These issues give rise to concern, but the accuracy of absorptiometry can be affected by the choice of calibrating materials. As a consequence, both absolute and relative values can differ substantially between manufacturers, between instruments and the ad hoc software used (Clasey et al., 1999; Prentice, 1995). Despite the multitude of DXA validation studies and despite the related controversy of its measuring quality, it is being reaffirmed that there have been comparatively few validation experiments of accuracy, and precision of either bone or BC measurements by cadaver and/or carcass analysis. More of these validations against direct values are necessary before we can be confident about the accuracy of absorptiometry (Prentice, 1995; Clarys et al., 2010b).

1.3 State of the art

A review of the state of the art of carcass studies related to DXA (Clarys et al., 2008, 2010b; Scafoglieri et al., 2010c) reveals validation attempts with rhesus monkeys (Black et al., 2001), mice (Brommage, 2003; Nagy & Clair, 2000), piglets (Chauhan et al., 2003; Elowsson et al., 1998; Koo et al., 2002, 2004; Picaud et al., 1996; Pintauro et al., 1996), pigs (Lukaski et al., 1999; Mitchell et al., 1996, 1998), pig hindlegs (Provyn et al., 2008), chickens (Mitchell et al., 1997; Swennen et al., 2004), and with dogs and cats (Speakman et al., 2001). The majority of these validation studies were based on chemical analysis and only a few on direct dissection comparison. Although almost all studies indicated perfect correlations for all variables with DXA, approximately half of the results of the various variables were found to be significantly different. In approximately a third of these studies, DXA was suggested to be valid and accurate for all its variables, whereas two studies indicated significant differences and/or erroneous data at all levels and for all variables. However, two important statements resulting from these studies are retained: (i) dissection and direct comparison combined with bone ashing are considered the most accurate and direct validation technique (Elowsson et al., 1998) and (ii) further research with direct dissection and ashing is needed (Prentice, 1995), in particular, with focus on the influence of abdominal and thoracic organs associated with dispersed gas/air pockets and internal panniculus adiposus (Provyn et al., 2008; Clarys et al., 2010b).

1.4 Aim

Although BC measurements by DXA are increasingly used in clinical practice, no study has been giving clarity yet about the content and meaning of “lean” as produced by DXA. Because direct dissection is the best possible direct measure, different soft tissue

combinations, e.g. skin, muscle, and viscera will be related to the DXA-lean variable. The exact knowledge of what is the content of the meaning of "lean" as measured by DXA is mandatory. In this chapter the reliability and the validity of Hologic QDR 4500/A DXA system will be determined based on direct *in vitro* measurements. A number of problems related to DXA applications resulting from BC terminology and data acquisition will be discussed.

2. Hologic QDR 4500/A DXA system

The DXA system is principally designed to provide information for the accurate diagnosis of osteoporosis, but also determines whole BC. A QDR 4500/A upgraded to Discovery Hologic DXA device (Hologic, Waltham, MA) (Figure1) utilizes a constant X-ray source producing fan beam dual energy radiation with effective dose equivalents (EDE) of 5μSv (e.g., to situate this low radiation in terms of example: a one-way transatlantic flight produces ±80μSv EDE and a spinal radiograph ~700μSv EDE) (Prentice, 1995). The estimations of fat and lean mass are based on extrapolation of the ratio of ST attenuation of two X-ray energies in nonbone containing pixels. The two X-ray energies are produced by a tungsten stationary anode X-ray tube pulsed alternately as 70 kilovolts (peak) (kVp) and 140 kVp. The software (for Windows XP version 12.4.3) performs calculations of the differential attenuations of the two photon energies and presents data of percentage of fat, fat mass (g), lean mass (g), BMC (g), BMD (g/cm^2), and total mass (g). According to the manufacturer, a coefficient of variation for human BMD of 0.5% can be expected during repeated measurements. DXA equipment was calibrated daily with a spine phantom (supplied by the manufacturers) to assess stability of the measurements, but also calibrated weekly using a step phantom to allow for correction of sources of error related to, e.g., skin thickness.



Fig. 1. Hologic QDR 4500/A DXA system

3. System reliability of DXA

Today it is generally accepted that using different models of DXA scanners (e.g. QDR 4500 Series against QDR 1000 Series), different software versions and different scanning speeds may reduce precision of body composition measurement (Barthe et al., 1997; Guo et al., 2004; Litaker et al., 2003)

Consequently the use of different DXA devices in longitudinal or multicenter studies may lead to measurement error and bias. On the other hand quality control studies proved that the intrainstrument, intra- and interoperator reliability for soft and rigid tissue mass estimates is high (Bailey et al., 2001; Burkhart et al., 2009; Glickman et al., 2004; Godang et al., 2010; Guo et al., 2004; Koo et al., 2002).

In order determine the reliability of the QDR 4500/A DXA system, 22 human cadavers (seventeen males and five females, mean age ± sd, 79.6 ± 8.8 y; mean weight ± sd, 69.589 ± 13.194 kg) were scanned three times consecutively. The first two scans were taken by a single experienced operator without repositioning the cadaver. From these data, the intramachine (test-retest) reliability for all DXA variables was calculated (Table 1). A third scan was taken and analyzed by a second experienced operator after repositioning the cadaver. This allowed for the calculation of the interrater reliability for whole BC assessment by DXA (Table 1).

The measurement results revealed no significant differences for the test-retest and between the operators, except for total mass. With coefficients of variation ranging from 0.2% to 3.5%, and with intraclass correlation coefficients ranging from 0.99 to 1.00 the Hologic QDR 4500/A DXA system showed to be highly reliable for BC assessment.

Variable	Reliability type	d ± sd	*P*	95% LOA	CV(%)	ICC
Total mass (g)	Intramachine	-49.70 ± 168.66	>0.05	± 330.6	0.2	1.000
	Interrater	-71.94 ± 162.38	=0.05	± 318.2	0.2	1.000
Fat (g)	Intramachine	-37.30 ± 338.67	>0.05	± 572.2	0.7	0.999
	Interrater	-132.71 ± 483.45	>0.05	± 912.4	1.0	0.999
Fat (%)	Intramachine	-0.02 ± 0.41	>0.05	± 0.81	1.9	0.999
	Interrater	0.11 ± 0.67	>0.05	± 1.31	3.5	0.998
Lean (g)	Intramachine	-10.77 ± 291.91	>0.05	± 663.8	1.9	0.999
	Interrater	58.34 ± 465.48	>0.05	± 947.6	3.5	0.999
BMC (g)	Intramachine	-1.63 ± 37.03	>0.05	± 72.5	1.6	0.997
	Interrater	2.43 ± 50.73	>0.05	± 99.4	2.4	0.995
BMD (g/cm²)	Intramachine	0.002 ± 0.019	>0.05	± 0.037	1.6	0.992
	Interrater	-0.014 ± 0.021	>0.05	± 0.040	1.8	0.990

Table 1. Intramachine and interrater reliability for whole body DXA variables (d = mean difference, sd = standard deviation, *P* = t-test significance level, LOA = limits of agreement, CV = coefficient of variation, ICC = intraclass correlation)

4. Validation of DXA for BC measurement

Twelve 6- to 18-month-old "Belgian Native" pigs were prepared for human consumption and were acquired within 2-day intervals, immediately after electroshock slaughter (six females and six castrated males, mean weight ± sd, 39.509 ± 4.335 kg). Special permission was obtained from the Belgian Directorate General of Public Health, Safety of the Food Chain and Environment, for the transport of the carcasses and for the nonremoval of abdominal and thoracic content, which is a normal procedure in consumption matters. The carcasses were exsanguinated and decapitated between the atlas and the occipital bone. To minimize further dissection error, front and hindlegs were disarticulated distal from humeri and femora, e.g., on elbow and knee levels, respectively. The mean weight ± s.d. of the

remaining carcass plus viscera was 33.051 ± 3.324 kg (whole carcass weights being taken with a digital hang scale (KERN-HUS-150K50) accurate to 50 g). The composition of the carcasses was studied in the following order.

4.1 Dissection procedure

After the DXA measurements, the carcasses were dissected into their various components as expressed on the tissue-system level: skin, muscle, AT, viscera, and bones (Wang et al., 1992). Muscle included tendon, blood vessels, and nerves belonging to the actual muscle. The subcutaneous, intramuscular (mostly intratendon), and intravisceral ATs were combined as one tissue. Again, blood vessels and nerves within AT were attributed to AT. Bones were carefully scraped, ligaments were added with muscle tendons to muscle tissue, and cartilage remained part of the bone tissue. Seven expert prosectors and anatomists worked simultaneously and each dissected particle was collected under cling film and kept in color-labeled, continuously covered plastic containers (12 × 10 × 10 cm) of known weight in order to minimize or eliminate evaporation (Clarys et al., 1999, 2010b; Provyn et al., 2008; Scafoglieri et al., 2010a). Full container mass was measured during the dissection by two researchers using Mettler Toledo digital scales (Excellence XS precision balance model 40025) accurate to 0.01 g. Once a bone was fully prepared, the same procedure was followed but completed with its hydrostatic weight while placed in a wire cradle suspended to the same scale allowing for the volume-based bone density (g/cm^3) calculation.

4.2 Chemical fat and hydration analysis

After the dissection and multiple weighing procedures, samples of all tissues of ~100–150 g (min–max) were deep-frozen. Small parts were cut off and weighed in recipients of known weight before lyophilization overnight. With dried samples, the water content was measured after storing into metal cells, and fat (lipids) extracted with technical hexane using a Dionex accelerated solvent extractor. After the hexane evaporation of the extraction, total (final) lipid content was determined (weighed). Part of the dissection protocol of the 12 porcine carcasses was the total defleshing of the skeleton, including the removal of extraosseous soft tendon and ligament tissue by scraping. Cartilage and intraosseous tissue (e.g., intervertebral discs) remained intact.

4.3 Ashing

The whole skeleton was diamond-cut into pieces in order to fit in the ashing furnace (type Nabertherm; Nabertherm, Lilienthal, Germany). After incineration, each sample was heated using a ramped temperature protocol of 2 hours to 800 °C and ashed for 8 hours, as determined by prior pilot work. Before weighing on the Mettler Toledo precision scale (accurate to 0.01 g), the ash was cooled undercover and collected in a main container. The ashing of one full porcine skeleton took between 50 and 60 hours.

5. Validity of DXA data acquisition

Although the interpretation of DXA results is generally straightforward, it is important to be aware of common pitfalls and to maintain rigorous quality assurance. The purpose of this part is to compare directly and indirectly obtained data of masses and densities (e.g., of whole body bone, adipose, and nonadipose tissue) using two different techniques and to provide information on the terminology as used in the respective methodologies.

5.1 Assumptions regarding data acquisition outcome

Table 2 shows an overview of terminology used per technique as applied and that are assumed to measure the same values. Although the basic assumption of equality of outcome and despite the different terminology used, knowledge of the ad hoc mass and density names will create a better understanding of the respective data acquisitions (e.g., Table 3).

Dissection	DXA	Biological background
Total mass (g)	Total mass (g)	-
Total tissue mass (g)	Total mass (g)	The Σ of all dissected tissue masses
Adipose tissue (AT)(g)	Fat (g)	AT is an anatomical issue FAT is a chemical issue (e.g. lipids)
Adipose tissue free mass (ATFM)(g)	Lean or lean body mass (LBM)(g)	ATFM is an anatomical concept LBM = Fat Free Mass plus essential lipids
Skeleton mass (g)	Bone mineral content (BMC)(g)	Skeleton or bone mass are morphological issues; BMC suggests the Σ of all mineral constituents of the skeleton
Ash weight (g)	BMC (g)	Ash weight = bone mass minus total bone hydration
Skeleton density (g/cm^3)	Bone mineral density (g/cm^2)	Volume (g/cm^3) based versus surface (g/cm^2) based density

Table 2. Different terminologies assumed to measure a similar outcome (DXA = dual energy X-ray absorptiometry)

Given the basic reasoning that the measurement of whole-body adiposity (in g or %), or nonadipose tissue (in g) and density (in g/cm^3) with different techniques using different equipment should produce similar, if not identical results on the same individuals, cannot be supported because of underlying assumptions, models, or approaches of the techniques and/or equipment are different (Beddoe, 1998; Heyward, 1996; Martin et al., 2003). This can be perfectly illustrated by the experiment on one single healthy male subject of which its % adiposity or fatness was measured with four different techniques on the same day, e.g., with an anthropometric formula, with dual energy X-ray absorptiometry, with bioelectrical impedance and with hydrostatic weighing including the calculation via the Siri (1956) formula. According to the original basic reasoning, the results of these four measures of adiposity should be in agreement. On the contrary, one notices with an anthropometric formula 12.5%, with DXA 17.5%, with bioelectrical impedance 21.5%, and with Siri 26.8% of adiposity was found (Table 3).

Method	Predicted whole body %fat
Anthropometry (Jackson and Pollock, 1978)	12.5
Dual energy X-ray absorptiometry	17.5
Bio-electrical Impedance Analysis	21.5
Hydrodensitometry (Siri formula)	26.8

Table 3. Predicted %fat by 4 different methods on one single male subject on the same day

5.2 Erroneous interchangeable use of BC terminology

The reality is that one is measuring different adiposity approaches with the same confusing terminology, e.g., % whole-body fat. Body fat (BF) is defined as the etherextractable constituent of body tissues, and must be considered as a chemical component of the body. This is already known since Keys and Brozek (1953). The interchangeable use of the terms BF and AT has led and is leading still to ambiguities and serious error. Among all DXA validation studies, only a few (Elowsson et al., 1998; Nagy & Clair, 2000) have defined the meaning of its adiposity variables mentioning or precising as DXA fat and lean against chemical (CHEM fat and CHEM lean).

In the Anglo-Saxon literature, in particular, the term "fat" is commonly used quantitatively when referring to the degree of obesity of a body and with "fatness" qualitatively referring to the appearance of the body that results from the deposition of AT (Wadden & Didie, 2003). Technically, "fat" may be defined biochemically as extractable lipid that consists of depot lipids such as the triglycerides and free fatty acids from AT and also so-called "essential" lipids such as structural phospholipids of cell membranes and nervous tissues, lipids of bone marrow, and a small moiety of other lipid-based compounds. Because of the confusion surrounding terms that are used both colloquially and technically, the terms "fat" and "fatness" should not be used. The term "fat" used in a biochemical sense should be replaced by the term "lipid," and the term "fatness" will be replaced by "adiposity" when referring to the quantity of AT in the body. ATs are masses separable by gross dissection and includes not just lipid but also the nonlipid constituents of cells, such as water and protein, and of course, the bulk of the subcutaneous AT and tissue surrounding organs, viscera and variable amounts between muscles, e.g., the intramuscular AT. These phenomena are known since 1950s and 1960s (Brozek et al., 1963) and were reinforced in 1980s by extensive direct data acquisition (Clarys & Marfell-Jones, 1986; Clarys et al., 1999). Table 2 indicates other discrepancies, e.g., for the nonadipose terminology. ATFM is an anatomical concept and lays in the continuation of the AT vs. FM. DXA pretends to measure lean or LBM as opposed to FFM, which could be expected because manufacturers claim to measure chemical components. In attempts to identify the physiologically relevant tissues, the concept of LBM was introduced more than half a century ago (Behnke et al., 1942). This consists of the FFM plus the essential fat specification that has varied from 2 to 10% for the FFM. Because of its imprecise definition, this term also has led to much confusion in the literature and is often erroneously used as a synonym for FFM. In addition to FFM and LBM, the anatomical concept of ATFM was proposed as a normalizing approach for interpopulation comparisons (Clarys & Martin, 1985; Clarys et al., 2010b).

5.3 Accuracy and precision of DXA data acquisition outcome

Table 4 combines the data acquisition of all directly obtained measures and the complete set of indirect estimates made by DXA. The purpose of this Table 4 is to evaluate the predictive quality of DXA, but also to evaluate precision and accuracy between direct and indirect values. For a good understanding and despite the significance of a correlation found, this study considers $r \geq 0.90$ as a good, $r \geq 0.80$ as a medium, and $r \geq 70$ as an average (mediocre) indicator of prediction. The t-statistics are considered as an indicator of precision or accuracy. Significant differences are set at $P < 0.05$. If not significantly different with the dissection reference, one can assume an acceptable level of measurement precision. Table 4 confirms that for almost all soft tissue (ST) comparisons, including total masses, a majority of good correlations ($r \geq 0.90$) and one medium correlation ($r \geq 0.80$) was found. Despite this

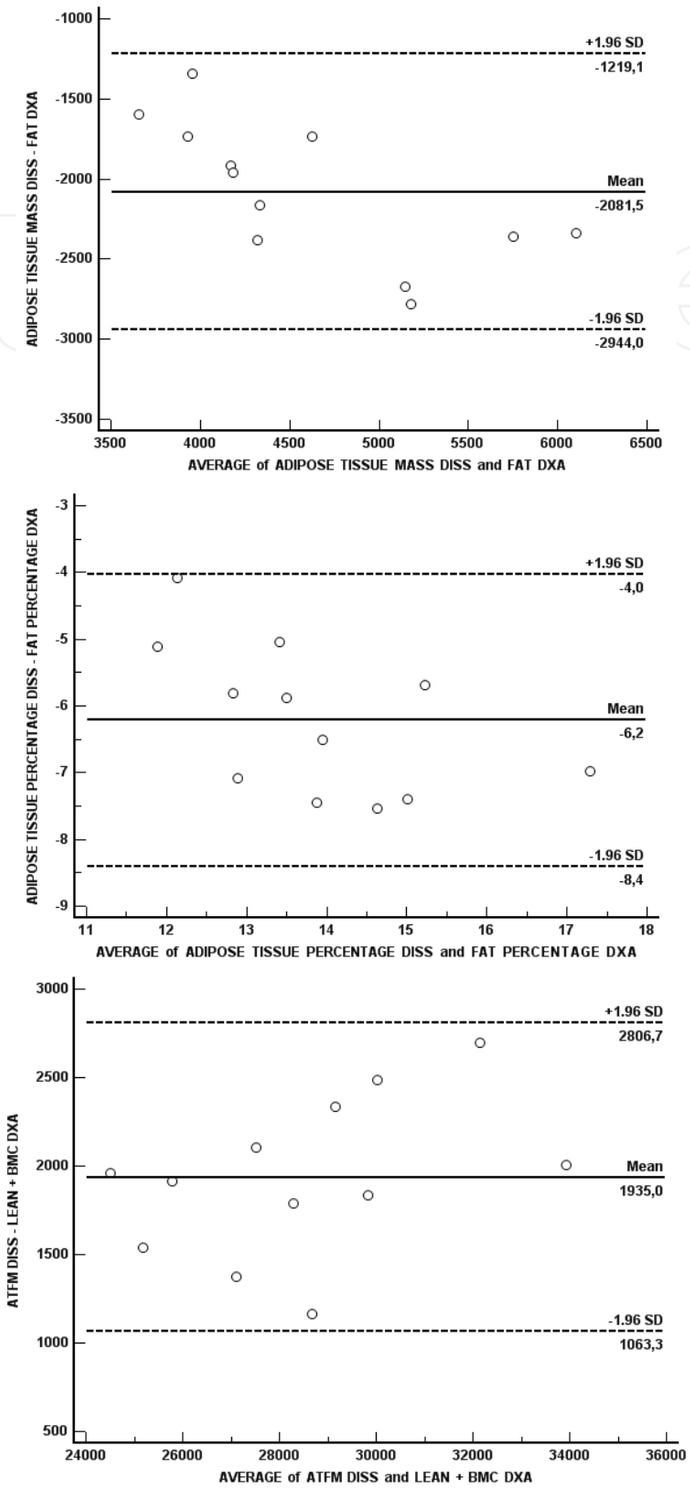


Fig. 2. Bland–Altman plots comparing adipose tissue (AT) and adipose tissue free mass (ATFM) by dissection to dual energy X-ray absorptiometry (DXA) measures with assumed similar outcome (BMC, bone mineral content; DISS, dissection)

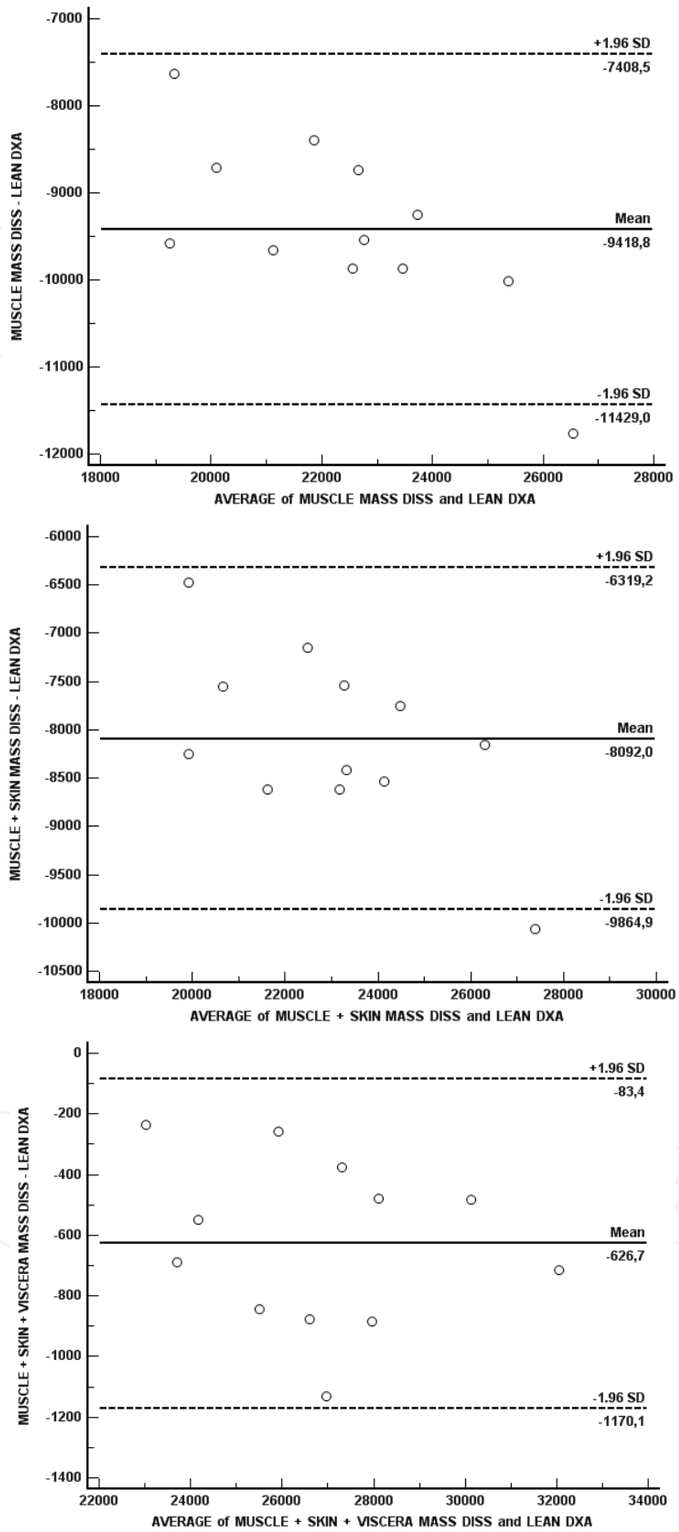


Fig. 3. Bland–Altman plots comparing "LEAN" by dual energy X-ray absorptiometry (DXA) to dissection measures with assumed similar outcome (DISS, dissection)

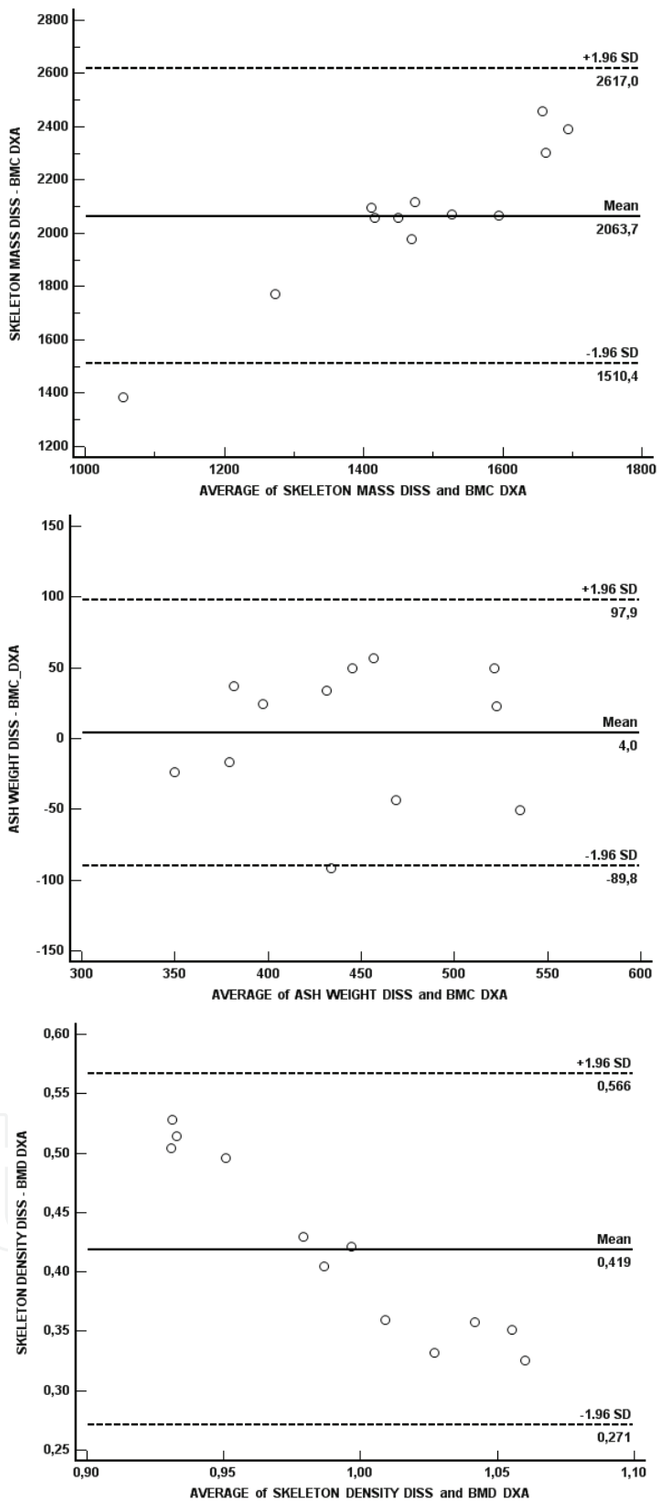


Fig. 4. Bland–Altman plots comparing different skeleton measures by dissection to dual-energy X-ray absorptiometry (DXA) measures with assumed similar outcome (BMC, bone mineral content; BMD, bone mineral density; DISS, dissection)

majority of good prognoses for prediction related to the dissection reference, we do find significant differences in accuracy for total masses, adiposity (g and %) for all nonadipose ST combinations, and for all bony comparisons except for the ashing which indicates an acceptable precision and comparability with DXA-BMC. Agreement and disagreement of DXA data acquisition with the dissection reference of the compared tissues and tissue combinations are shown in Figures 2–4 with 9 Bland–Altman (1986) plots. The solid lines indicate the mean difference deviation from zero and the dashed lines the individual variation between ±1.96 s.d.

Variable	Dissection x ± sd	DXA x ± sd	r	*P*
Total mass (g)	33051.3 ± 3323.8	33192.3 ± 3336.6	1.00	<0.01
Total tissue mass (g)	32723.4 ± 3427.0	33192.3 ± 3336.6	1.00	<0.001
Adipose tissue/Fat (g)	3571.6 ± 632.8	5653.1 ± 934.1	0.91	<0.001
Adipose tissue/Fat (%)	10.8 ± 1.27	17.0 ± 1.87	0.81	<0.001
ATFM/Lean + BMC (g)	29479.7 ± 2874.7	27544.7 ± 2681.5	0.99	<0.001
Muscle/Lean (g)	17684.3 ± 1908.8	27103.1 ± 2647.3	0.95	<0.001
Muscle + skin/Lean (g)	19011.1 ± 2092.3	27103.1 ± 2647.3	0.95	<0.001
Muscle + skin + viscera/Lean (g)	26476.4 ± 2593.8	27103.1 ± 2647.3	0.99	<0.001
Skeleton mass/BMC (g)	2505.3 ± 317.5	441.6 ± 64.6	0.62	<0.001
Ash weight/BMC (g)	445.6 ± 66.2	441.6 ± 64.6	0.73	NS
Skeleton density(g/cm^3)/BMD(g/cm^2)	1.201 ± 0.02	0.782 ± 0.09	0.68	<0.001

Table 4. Comparison between direct dissection data values with the corresponding DXA values (DXA = dual energy X-ray absorptiometry, x = mean, sd = standard deviation, r = Pearson correlation coefficient, *P* = t-test significance level, ATFM = adipose tissue free mass, BMC = bone mineral content, NS = not significantly different)

The Bland–Altman plots confirm the findings as shown in Table 4. If we look at the mean value level of the respective variables, there cannot be any doubt that DXA produces anatomical–morphological quantities, evidently at all adipose and nonadipose combinations. (Clarys et al., 2010b)

5.4 Assumptions regarding chemical tissue composition constancies

The chemical tissue composition of the dissection masses was determined according to anatomic segmentation into upper limb, lower limb, and trunk (e.g., for skin, muscle, and bone). For AT, additional differentiation was made for subcutaneous (e.g., external) and visceral (e.g., internal) trunk AT. For each segment, the water content and the fat (e.g., lipid) content was determined for the respective tissues and presented as % of the studied mass per tissue in Table 5.

With the confounding effect of the high variability of AT removed, the composition of the ATFM shows smaller deviations of its components and smaller differences between males and females than when body mass is used as a reference (Martin & Drinkwater, 1991). Conversion from FFM to LBM and/or to ATFM is susceptible to significant error because we are dealing with two totally different models. In addition, DXA does not take into account the water content and lipid content variations (Table 4) of both its adipose and nonadipose constituents. Small variation of tissue hydration may explain important differences of ad hoc estimates (Prior et al., 1997; Wang et al., 1999, 1995). In DXA, and other newer technologies (Muller et al., 2003), body fat is calculated on the constancy assumption

that ≈73% of LBM (e.g., lean or lean + BMC) is water (31). This assumed constancy of hydration, e.g., the observed ratio of total body water to FFM was confirmed in humans by Wang *et al*. (1999). However, this assumption is subject to some questions that highlight the need for more research on the matter. Viewing tissue water content obtained by lyophilization in several human tissue studies, one can make two observations: (i) assuming a constant % of water in FFM may be jeopardized by the variable tissue water content within and between the tissues that compose FFM; and (ii) water content in AT is highly variable, e.g., ranging from ±17% to ±50% in humans (Table 6) (Provyn et al., 2008; Clarys et al., 2010b).

Tissue	Segment	Water content(%) x±sd	Lipid content(%) x±sd	r
Skin	Up limb	61.0 ± 8.6	4.6 ± 6.0	- 0.73
	Lo limb	60.7 ± 4.9	4.3 ± 1.4	- 0.55
	Trunk	50.1 ± 9.3	10.2 ± 7.4	- 0.20
Adipose	Subcut Up limb	47.2 ± 7.0	15.0 ± 7.0	- 0.72
	Subcut Lo limb	47.2 ± 6.6	15.6 ± 6.9	- 0.84*
	SubcutTrunk	21.0 ± 5.3	29.0 ± 7.3	- 0.16
	Visceral Trunk	50.1 ± 10.6	19.0 ± 6.7	- 0.70
Muscle	Up limb	75.4 ± 1.4	1.4 ± 1.0	- 0.86*
	Lo limb	74.5 ± 2.7	3.1 ± 3.2	0.16
	Trunk	73.8 ± 3.9	3.7 ± 2.3	- 0.70
Bone	Up limb	39.0 ± 8.2	10.9 ± 2.7	- 0.84*
	Lolimb	39.5 ± 8.1	9.8 ± 1.9	- 0.71
	Trunk	49.4 ± 2.4	7.7 ± 3.3	- 0.20

Table 5. Water (lyophilization) and lipid (ether extraction) content of different tissues and relationship (x = mean, sd = standard deviation, r = Pearson correlation coefficient, Up = upper, Lo = lower, Subcut = subcutaneous, *P<0.01) (Clarys et al., 2010b)

	Muscle	Skin	Viscera	Bone	AT
Forbes & Lewis (1956) (n=2)	1) 67.5	53.7	73.4	26.8	26.2
	2) 68.2	51.8	72.0	31.6	18.3
Mitchell et al. (1945) (n=1)	79.5	64.7	76.6	31.8	50.1
Cooper et al. (1956) (n=2)	1) 68.9	53.5	73.7	30.2	16.8
	2) 77.3	72.5	77.8	39.5	83.9
Forbes et al. (1953) (n=1)	70.1	57.7	73.3	28.2	23.0
Clarys et al. (1999) (n=6)	70.8	63.2	79.1	---	21.6

Table 6. Water content (%) of lean and adipose tissue masses in humans (lyophilisation) (AT = adipose tissue)

This is confirmed in this study on animal corpses with % whole-body water content ranging from ±20 to ±50% (Table 5) repeating that the constancies claimed by DXA cannot be maintained (e.g., with fluid ranging between ±50 and ±61% for skin, between ±39 and ±49% for bone, but little variability for muscle). Because no total tissue lipid extraction was carried out because technical circumstances allowed sample fractionation only, lipid content is expressed as % of the measured sample mass. From sample masses being identical for hydration and lipid fractionation (Table 5), we learn that lipid content of tissues is variably

related to its ad hoc fluid content, but if the extremities are considered separately, one notices an apparent constancy both in hydration and lipid fractionation. The fact that all trunk tissue data (e.g., in skin AT, muscle, and bone) deviate both, but nonsystematically in hydration and lipid content from the upper and lower extremities indicate the importance of the trunk as discriminating segment and the associated abdominal/metabolic syndrome theories. As Elowsson *et al*. (1998) and Provyn *et al*. (2008) were previously evaluating the accuracy of DXA with dissection in animals, both studies motivated the choice of using plain carcasses (decapitated pigs without abdominal and thoracic organs) or just hindlegs to minimize various errors. According to Elowsson *et al*. (1998), with DXA this would marginally increase DXA's underestimation. This can no longer be supported; on the contrary, not measuring the internal trunk will just increase the error because of an assumption of segment constancy of hydration and ad hoc lipid fractionation. Wang *et al*. (1999) examined *in vitro* and *in vivo* studies allowing a review and critical appraisal of the importance of hydration of FFM and confirming the findings of Provyn *et al*. (2008). They conclude that, even though methodological limitations preclude a highly accurate analysis, adult mammals, including humans, share in common, a relatively constant hydration of FFM. The segmental data presented in Table 5 within a four-component dissection model dismisses the idea of constant hydration of FFM. In addition, the assumed ad hoc constancy of 0.73 cannot be retained. The question whether the hydration status of FFM, LBM, or ATFM reflects physiologic regulatory mechanisms (Going et al., 1993; Wang et al., 2005) cannot be answered, but it seems that trunk nonadipose tissues may affect hydration differently than the lean tissues of the extremities or vice versa (Table 5). (Clarys et al, 2010b)

5.6 What does DXA measure?

Regardless of the existing mechanisms and regardless of the hydration and lipid (fat) content of nonadipose tissue, this study has not been able to detect what the content is of the DXA nonadipose variables, e.g., "lean" and/or "lean + BMC." We still do not know what DXA is exactly measuring under these ad hoc headings. "Lean" compared with muscle tissue, with muscle plus skin tissue, and with muscle plus skin plus viscera (dissection) resulted in equally high correlations (r values between 0.95 and 0.99) assuming a good prediction estimate but with systematic significant difference confirming its imprecision, "lean plus BMC" is certainly not measuring ATFM (e.g., skin + muscle + viscera + bone), although its high $r = 0.99$, but again with a significant difference ($P < 0.001$) indicating a lack of precision and accuracy. Contrary to Bloebaum *et al*. (2006), but in agreement with Louis *et al*. (1992), BMC seems a good estimate ($r = 0.73$) with no significant difference of its ash weight. The impression is given, however, that DXA nonadipose values are expressed as anatomical–morphological values combined with chemical elements. This study cannot confirm what the nonadipose component of DXA is measuring, but it does confirm that all the DXA components are subject not only to measurement error but also to terminology error and violation of basic assumptions. It is known since many decennia that density in its weight/volume quantification (g/cm^3) can be considered as an additional and separate dimension of BC. The DXA-derived BMD, however, is a weight/surface quantification (g/cm^2) and therefore not a true density, nor the density based on which indication of osteoporosis classifications were studied in the past (Bolotin, 1998, 2007; Bolotin & Sievanen, 2001; Bolotin et al., 2001; Lochmuller et al., 2000). In a pilot (dissection) study using porcine hindlegs in which DXA BMD was compared with bone covered with muscle, AT, and skin tissue, and compared with scraped bones only (Clarys et al., 2008; Provyn et al., 2008), it was found that DXA BMD underestimates true density with more than 40%. In this study (Table

4), under whole-body conditions, one notices a similar level of high underestimation of DXA but with a better correlation, e.g., $r = 0.68$ for the whole-body value against $r = 0.39$ for the hindleg study. The extensive work done by Bolotin (2007) shows DXA-measured BMD methodology (*in vivo*) to be an intrinsically flawed and misleading indicator of bone mineral status and an erroneous gauge of relative fracture risk. The transfer of their findings to the *in situ* carcass situation of this study confirms that the DXA methodology cannot provide accurate, quantitatively precise, meaningful determinations of true bone densities and proper bone mass because of the contamination of independent ST, e.g., fluid and lipid content contributions. (Clarys et al., 2010b)

6. Conclusions

The majority of present consensual acceptance and understanding of the DXA estimate quality rests solely upon a number of well-established, multiconfirmed, *in vivo* and *in situ* significant high correlations. In terms of true "reality precision" measures, DXA produces inaccurate and misleading values at all levels of its output. Both the adipose and nonadipose components of DXA ignore the ad hoc lipid content, and the nonadipose variables do not take into account the true composing tissues. Lean and lean + BMC of DXA do not correspond to anatomical–morphological tissue combinations, nor to chemical values. It cannot be determined what DXA really measures. Bone mineral content versus ash weight is the single variable with a close reality and nonsignificant difference output. DXA is based on a series of constancies within tissues, regardless of segments, hydration, and lipid content variability. These results suggest that clinical precision essential in individual diagnosis of health status is at risk. Erroneous terminology, constancy assumptions related to hydration and lipid distribution, and two-dimensional data acquisition principles are the major causes of these problems. The hypothesis that DXA methodology provides accurate, precise, and relevant BC determinations is proven to be unwarranted and misplaced.

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