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Critical Appraisal of Data Acquisition in Body Composition: Evaluation of Methods, Techniques and Technologies on the Anatomical Tissue-System Level

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1. Introduction

Human body composition (BC) could well be the only health-related discipline that allows both "simple indexes" e.g. the Body Mass Index, and "advanced technology" e.g. dual energy X-ray absorptiometry, to be used for the same purpose: the quantitative measurement of BC components for the prediction of health risk. Human BC probably is the most confusing health-related discipline also because of the mixture of apparent corresponding and analogue terminology. The major problems with quality control in BC are the large amount of different models and devices each with their own advantages and limitations, and their respective validation against other indirect data-acquisition methods or technologies. The validation of anthropometric techniques has generally been performed against two- or three-compartment models of BC such as hydrodensitometry or dual energy X-ray absorptiometry. These reference standards themselves are based on assumptions of a constant density, constant hydration and constant tissue distribution throughout the human body, without taking into account the biological variation of tissue composition. The quality control and validation of indirect *in vivo* measurements for BC against direct measurements is both scarce/almost un-edited and essential: it is known that there are wide variations in the indirect measurements currently used in practice, which makes any clinical decisions based on those measurements suspect. This chapter will give a critical appraisal of three frequently used data acquisition methods for measuring individual BC, in particular hydrodensitometry, body mass index and dual energy X-ray absorptiometry. In addition this chapter will provide a (renewed) critical appraisal of the most confusing terminology.

2. The hazards of hydrodensitometry

The human body composition can be considered according to different situations, within different disciplines and based on different theories, e.g. clinical versus biological approaches, chemical versus anatomical evaluations, *in vivo* versus *in vitro* research, direct

data acquisition versus indirect prediction techniques, BC in ergonomics or BC in health sciences, etc...Most of these differentiations can be found in the respective 2-, 3-, 4- and 5-component (2C, 3C, 4C and 5C) models (Table 1) that have been described since the first model, e.g. the 4C model of Matiegka (1921).

| | | | | | |
|-------------------------------------|----------------|--------------------------|---------------------|----------------------|----------------------|
| Model 2C Siri (1956) | Fat | Fat Free Mass | | | |
| Model 2C Clarys & Martin (1985) | Adipose Tissue | Adipose Tissue Free Mass | | | |
| Model 3C Siri (1961) | Fat | Body Water | | Density | |
| Model 3C Mazess et al. (1981) | Fat | Lean Body Mass or Lean | | Bone Minerals | |
| Model 4C Widdowson et al. (1951) | Fat | Body Water | Proteins | Minerals | |
| Model 4C Matiegka (1921) | Adipose Tissue | Skeletal Muscle | Bone | Residual Lean Mass | |
| Model 4C Lohman & Going (1993) | Weight | Body Water | Bone Minerals | Density | |
| Model 5C Clarys et al. (1984) | Adipose Tissue | Skin Tissue | Muscular Tissue | Bone Tissue | Organ Tissue |
| Model 5L Wang et al. (1992) | Atomic | Molecular | Cellular | Tissue/Organ | Whole Body |
| Model 5C Wang et al. (1992) | Fat | Extracellular Fluid | Intracellular Fluid | Extracellular Solids | Intracellular Solids |

Table 1. Theoretical multi-components models used in body composition analysis (C = Component, L = Level)

Partly in parallel and partly as a consequence of the development of these models, terminology and an associated confusion has installed itself over the years in particular within the (almost) colloquial use and mixing of the terms fat, adipose tissue (AT), lipids, fat free mass (FFM), lean and lean body mass (LBM) or adipose tissue free mass (ATFM). This multitude of related terminology has led to a proliferation of the confusion without reflecting on the absolute direct and indirect underlying values. This was already predicted in the sixties (Behnke, 1963) but confusion increased nevertheless. Chemical and anatomical analyses produce direct values and were originally applied in *in vitro* studies only (Brodie et al., 1998; Clarys et al., 1984, 1999; Forbes & Lewis, 1956; Forbes et al., 1953; Marfell-Jones et al., 2003; Roemmich et al., 1997). With the development of techniques, methods and equipment, magnetic resonance imaging (MRI) and computed tomography (CT) produce data that are closest to real data acquisition allowing comparison with what is considered as “direct” values (Janssen et al., 2002a; Mitsiopoulos et al., 1998).

2.1 Limitations and restrictions of hydrodensitometry

The term direct data acquisition is valid also for the volume measurements in plethysmography and water displacement. Hydrostatic weighing including the ad hoc density calculation is a direct value also but the next step allowing quantifying fat from it certainly is not a “direct” value. Apart from the direct underwater weighing, hydrodensitometry (HD) needs equally a direct measurement of residual lung and gastro-intestinal air, realizing that small errors in residual volume may create more important errors in the subsequent calculations. In addition, it needs to be reminded also that this

measurement series is not evident with children, older individuals and persons (all ages) with aquaphobia. Hydrodensitometry is an essential part of the 2C model and has served as a direct “reference method” for many (if not most) BC techniques and methods (Brodie et al., 1998; Brozek et al., 1963; Ellis, 2000). This model is based on the assumption of a density constancy of 0.901 g/ml for fat and of 1.100 g/ml for FFM and, irrespective the technique or method it was used for, the background of constancy was projected to all tissues that compose FFM and irrespective the measurement of fat (Behnke et al., 1942; Siri, 1956). Although this knowledge, in part or as a whole, has been extensively described and discussed in the literature, one must ascertain that HD has not been abandoned as a reference and is used in various sport medical centers and sport science laboratories still (Brodie et al., 1998; Eston et al., 2005; Westerterp et al., 2008; Wouters-Adriaens & Westerterp, 2008). Readers are reminded that distinction needs to be made between HD for the purpose of %fat determination and for the purpose of single tissue density measurement. The first is a direct measure plus an indirect calculation, the latter remains direct to obtain density only, without associated problems. In general, the studies evaluating the quality of HD using an *in vivo* 4C model as the criterion have shown that estimation of body density accurately estimates the mean body composition of most groups of non-athletic adults e.g. black, elderly and obese subjects and its accuracy is not specifically related to race, age, gender or level of adiposity (Visser et al., 1997). However, various degrees of inaccuracy exist and systematic error is substantial for most individuals and groups of children, adolescents and athletes. In other words, the possibilities and limitations are or should be known and more definitive reviews and studies quantifying the inaccuracy of the method are available (Brodie et al., 1998; Clarys et al., 2010a; Ellis, 2000; Lohman, 1981; Modlesky et al., 1996; Roemmich et al., 1997; Visser et al., 1997). The cause of these inaccuracies is complex and not only a reflection of musculoskeletal development (Prior et al., 2001). Directly obtained data combined with known information could provide the missing links for this matter.

The purpose of this chapter section is to combine well known evidence with new direct data acquisition and analyses to accept or reject HD and the 2C model as a measure and as a reference.

2.2 Methodology

The bases for data collection is of an anthropological nature. Data sources are used from the 19th and 20th century and completed with the Brussels Cadaver Analysis Studies (BCAS) (Clarys et al., 1999). This data collection is partly projected on and combined with the basic plot of Siri's equation (Figure 1) theories to predict fat from whole body density within the 2C model (fat + FFM) (Siri, 1956).

Methodology varied across the different studies, depending on the objectives, but these differences would have little effect on the measured weights of the gross tissues.

Pooling of all Brussels data yielded a dataset of 34 cadavers (17 male and 17 female) with an age range 16-94 yrs. The 19th century data consisted of 12 subjects, age range 26-50 yrs (though four subjects were of unknown age). Adding the remaining five dissections of the mid 20th century yielded a total of 31 men (mean age \pm sd; 56,6 \pm 21,5y) and 20 women (mean age \pm sd; 75,5 \pm 15,4y) for whom the weights of skin, adipose tissue, muscle, and bone were known. Heights were not reported for three individuals. Detailed data are to be found in Clarys et al. (1999). In addition the *in vivo* HD literature was screened for biologically debatable data obtained within the 2C model, for example unrealistically low estimates of body fat (Adams et al., 1982; Katch & Michael, 1968; Pollock et al., 1977).

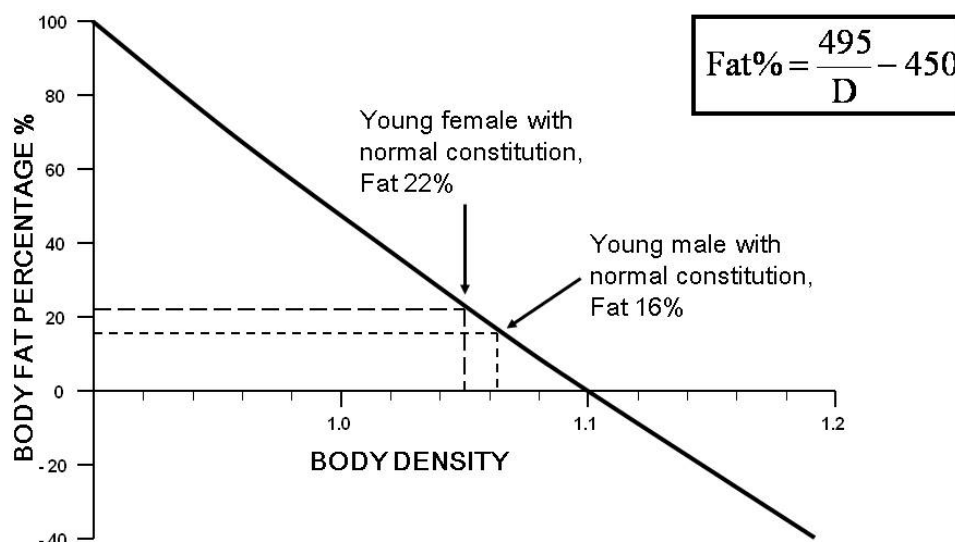


Fig. 1. Siri's plot (1956), the base of hydrodensitometry for the prediction of the percentage of body fat

2.3 Critical appraisal of body composition terminology

The densitometric analysis of BC has been limited in regard to its validity, by the absence of direct information of the fat content and densities of known bodies, segments and tissues. In attempts to identify the physiologically relevant tissues, the concept of the LBM was introduced more than half a century ago (Behnke et al., 1942). This consists of the FFM plus the essential lipids whose specification has varied from 2 to 10% for the FFM (Brodie et al., 1998; Keys & Brozek, 1953). Because of the imprecise definition of LBM, this term has led to much confusion in the literature and is often erroneously used as a synonym for FFM. The fat and FFM are chemical concepts without anatomical or physiological basis. It is composed of the fat-free tissues and fat-free fluids of the body. For its density to be constant requires that both the following conditions be satisfied simultaneously: 1) the proportions of all the fat-free tissues (FF muscle, FF bone, FF skin, FF organs, etc.) must be fixed and thus constant; and 2) the densities of these fat-free components must be constant.

In addition to FFM and LBM, the anatomical concept of ATFM (Table 1) was proposed as a normalizing approach for interpopulations comparisons (Clarys & Martin, 1985). 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). Changing from FFM to LBM and/or to ATFM is susceptible to significant error (Clarys et al., 2010a). As a result and within the 2C models fat + FFM and AT + ATFM we are dealing with two totally different models ... a chemical 2C model and an anatomical 2C model (Figure 2). Pragmatic observers may recognize the 4C model of Widdowson et al. (1951) and the 4C or 5C models of Matiegka (1921) and Clarys et al. (1984) as respectively chemical and anatomical alternatives also.

To illustrate some of the problems caused by the failure of these assumptions and as a result of the terminology confusions Table 2 shows the results of so-called "chemical" %fat determinations on one single male subject on the same day. These results should have been identical or at least similar between methods and compared to the HD reference, but aren't. Some methods, indeed may suggest "chemical fat = lipids", e.g. dual energy X-ray

absorptiometry, but other methods, e.g. Jackson and Pollock’s formula (1978) with its anthropometrical parameters suggest the measurement of AT. Finally, bio-electrical impedance analysis (BIA) and HD are nor chemical nor anatomical techniques.

| Chemical 2C model | Anatomical 2C model |
|---|--|
| <div>Fat (= lipids)</div> <div>+</div> <div>Fat Free Mass (FFM) (= fluids + proteins + body minerals)</div> | <div>Adipose tissue (= subcutaneous + intramuscular + internal)</div> <div>+</div> <div>Adipose Tissue Free Mass (ATFM) (= skin + muscles + bone + organs)</div> |

Fig. 2. Two-component models ... two different worlds

| Method | Predicted whole body %fat |
|---|---------------------------|
| Anthropometry (Deurenberg et al., 1991) | 25.1 |
| (Jackson and Pollock, 1978) | 12.1 |
| Dual energy X-ray absorptiometry | 17.5 |
| Bio-electrical Impedance Analysis | 21.5 |
| Hydrodensitometry (Siri formula) | 26.8 |

Table 2. Predicted %fat by 4 different methods on one single male subject on the same day

2.4 Variation of bone density and water content of body tissues

It needs repeating that HD does not take into account density differences and water content variations of its lean tissues (fat-free or adipose-free). Bone is the tissue with the highest density and varies physiologically with age and exercising habits, while water is the element fluctuating most within the lean tissue component (Clarys et al., 1999, 2005, 2010a; Johansson et al., 1993). In HD, but also in newer technologies, e.g. DXA body fat is calculated on the constancy assumption that ~73% of LBM is water (Brommage, 2003; Clasey et al., 1999; Eston et al., 2005; Prior et al., 1997). This assumed constancy of hydration e.g. the observed ratio of total body water to FFM was confirmed 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 (TWC) obtained by lyophilisation (Table 4) in several studies, one can make two observations: 1) assuming a constant % of water in FFM may be jeopardized by the variable TWC within and between the tissues that compose FFM; and 2) water content in AT is highly variable e.g. ranging from ±17% to ±84%. The studies of Wang et al. (1995, 1999) did indeed raise the question “Does body adiposity influence hydration?”. The data in Table 3 and 4 clearly suggest that whatever constancy claimed it cannot be maintained. Component models should allow for hydration variability. According to Fogelholm et al.

(1997) knowledge of the hydration household is recommended when assessing changes in BC during substantial weight reduction.

| | 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 3. Water content (%) of lean and adipose tissue masses in humans (lyophilisation)

Table 4 on the other hand, lists direct mean densities of separate bones of the body. Different bone dimensions, a maximum difference between bones of 0.406 g/ml and all densities superior to 1.100 g/ml will influence the density of the LBM. No doubt that the amount of variance shown will lead equally to serious error in the densitometric prediction of body fat (Clarys et al., 2010a).

| Bone | Density (g/ml) mean ± sd |
|---------------------|--------------------------|
| Pelvis | 1.164 ± 0.037 |
| Tibia | 1.242 ± 0.055 |
| Humerus | 1.262 ± 0.054 |
| Femur | 1.267 ± 0.053 |
| Clavicula | 1.315 ± 0.058 |
| Radius | 1.353 ± 0.070 |
| Ulna | 1.395 ± 0.078 |
| Cranium | 1.403 ± 0.061 |
| Mandibula | 1.570 ± 0.100 |
| Mean separate bones | 1.330 ± 0.053 |
| Whole skeleton | 1.236 ± 0.039 |

Table 4. Mean densities (g/ml) of separate bones and of the whole skeleton (n=25)

The step from “error” to “hazard” is not too difficult and for the purpose we have reproduced the basic plot of Siri’s equation (Figure 1) allowing for the prediction of fat. Applying the values $\rho_{FFM} = 1,100$ g/ml and $\rho_{Fat} = 0,901$ g/ml, one recognizes the well-known equation of Siri (1956). The plot of this equation shown in Figure 1 is projected beyond the normal range (0,901-1,100) for whole body density. Typical fat values for young males (16%) and young females (22%) are illustrated. The negative region indicates clearly that if whole body densities greater than 1,100 g/ml are observed then negative values of body fat will be predicted. The occurrence of such values would be a clear indication of violation of the assumption of constant density for the FFM. Negative values of body fat presumably have been omitted as erroneous in the past and one can assume that many of these data never were published. A review of the literature, however, reveals a few studies showing these anomalous fat estimations. Repeating these data will complete the whole

picture as stated in the purpose. In a study of 20 elite middle-distance and marathon runners, Pollock et al. (1977) found that 5 had predicted body fat less than 2%. The leanest of these had a total estimated fat content of 120 g, less than the amount typically found in the brain or vertebral column alone. In addition, this subject displayed measurable subcutaneous AT since the sum of the 7 skinfolds taken was 31,5 mm, an average of 4,5 mm per skinfold. This average corresponds with the absolute maximum skin thickness to be found in the shoulder region (Clarys et al., 2005). In young males, body densities of 1,102 g/ml and 1,104 g/ml have been measured, corresponding to -0,8% and -1,6% fat, respectively (Behnke et al., 1942; Katch & Michael, 1968). However, it is for lean mesomorphs that the most anomalous results have been found. In a study of 29 Canadian professional football players, Adams et al. (1982) found that 12 had predicted fat (from density) less than 2%. Of these, nine had an estimated amount of fat ranging from zero% fat to -12% fat. Table 5 shows whole-body density, predicted fat percentage and the sum of 10 skinfolds for each of these 9 subjects.

| Subject number | Body density (g/ml) | %fat (Siri's equation) | Sum of 10 skinfolds (mm) |
|----------------|------------------------|---------------------------|-----------------------------|
| 22 | 1.100 | 0.0 | 63 |
| 16 | 1.101 | -0.4 | 74 |
| 24 | 1.102 | -0.8 | 57 |
| 2 | 1.103 | -1.2 | 55 |
| 5 | 1.103 | -1.2 | 97 |
| 9 | 1.105 | -2.0 | 69 |
| 26 | 1.105 | -2.0 | 87 |
| 28 | 1.129 | -11.6 | 64 |
| 25 | 1.130 | -12.0 | 88 |

Table 5. Negative body fat predictions for 9 professional football players (Adams et al., 1982)

Evidence was presented that the variations in bone density and H₂O content of tissues in the composition of the FFM are considerably greater than suggested by previous researchers. For a lean male of whole body density 1,100 g/ml, a measurement deviation as small as ±0,020 g/ml leads to percent fat predictions ranging from +8,3% to -8,0%. This variability explains the inaccuracy of body fat predictions observed by some researchers when applying conventional HD.

2.5 Validity of hydrodensitometry

In combination with the subject's cooperation dependence, its related error allowance, whole body density and the derived %fat is affected with a serious violation of its basic assumptions and can no longer be considered as valid, not to mention as a criterion measure. Whole body density may not be confounded with single tissue density, which remains a useful biological parameter since it produces indication of changing tissue proportions and ad hoc density patterns within the body. Consideration should be given to the undeniable fact that many if not most *in vivo* whole body fat and AT determination methods have been validated with HD. For the practitioner or the research laboratory that does not have the resources to have higher order models or imaging possibilities such as MRI or CT, the acceptance and enforcement of the hypothesis that HD and the 2C model are

not appropriate and that consequently it should be banned from operation is a too strong message that may not be extrapolated to predictive survey research and may not suggest that all methods and techniques derived or validated against HD are also invalid and obsolete (Clarys et al., 2010a).

3. Controversial use of body mass index in longevity

The body mass index (BMI) and waist circumference (WC) are parameters used in the screening for and classification of overweight and obesity in adult individuals, based on their respective (apparent) correlation with total body and visceral adiposity (NHLBI, 1998; WHO Obesity, 2000). These relationships, however, are based on indirect estimations of adiposity and/or other prediction values (Baumgartner et al., 1995; Clarys et al., 1999; Scafoglieri et al., 2009). In fact, the validation of BMI and WC as indicators of adiposity has principally been performed against 2C or 3C models of BC such as HD, BIA or DXA. These reference standards are based on predictive equations that assume constancy and/or homogeneity of the compartments without taking into account the human biological variation of tissue composition (Clarys et al., 2009, 2010b; Deurenberg, 2003; Heymsfield et al., 1997; Scafoglieri et al., 2009, 2010). Ideally, validation as markers of adiposity should be performed against multi-compartment models of BC as provided by three-dimensional imaging techniques such as CT and MRI or against direct measurements of AT such as total body carbon assessment and whole-body dissection (Heymsfield et al., 1997; Kvist et al., 1988; Ludesher et al., 2009). Even though CT and MRI are often cited as *in vivo* reference standards for the quantification of tissue-system level components, publications describing validation of these techniques with human cadavers remain scarce (Abate et al., 1994; Foster et al., 1984; Janssens et al., 1994; Mitsiopoulos et al., 1998; Rossner et al., 1990; Scafoglieri et al., 2010).

Emerging evidence indicates that health-related assessment of BC in the elderly is more appropriate if muscle mass and adiposity are considered jointly, instead of separately (Rolland et al., 2009; Zamboni et al., 2008). In this context, BMI has been suggested as a powerful indicator of muscle mass in elderly persons (as determined by DXA) (Iannuzzi-Sucich et al., 2002). Sarcopenia, defined as age-related loss of skeletal muscle mass, creates a major BC change that contributes to a large percentage of disability with increasing age (Bautmans et al., 2009; Janssen et al., 2002b). In parallel, ageing is accompanied by an increase in visceral adiposity, which is a known risk factor for morbidity and mortality, even when the total amount of AT remains constant (Zamboni et al., 1997). Because AT replaces lean tissue with increasing age, older subjects tend to present a greater proportion of AT compared to younger individuals with the same BMI (Baumgartner et al., 1995; Elia, 2001).

It remains unclear how BMI and WC relate to BC measures in the elderly. Therefore the aim of this chapter section is to explore the relationship of BMI and WC with important metabolic tissues masses, in particular with muscle tissue mass, with adipose tissue mass, with muscle/AT mass ratios and with trunk adipose tissue distribution, based on an anatomical 5C model obtained by dissection of cadavers of elderly persons.

3.1 Methodology

By means of a will system, adult Belgian citizens can donate their bodies for medical and scientific research purposes to the university of their choice. All data were collected in the Department of Anatomy at the Vrije Universiteit Brussel (Brussels, Belgium) during

separate whole-body dissection projects known as the BCAS (Clarys et al., 1984, 1999; Janssens et al., 1994). The most common cause of death of the subjects was heart disease (Table 6).

| | Female (n = 17) | Male (n = 12) |
|---------------------------|-----------------|---------------|
| Natural | 5 | 6 |
| Heart attack | 6 | 4 |
| Stroke | 1 | 0 |
| Accident | 1 | 0 |
| Cancer | 2 | 1 |
| Renal insufficiency | 1 | 0 |
| Respiratory insufficiency | 0 | 1 |
| Leukemia | 1 | 0 |

Table 6. Causes of Death of the Subjects (official diagnose on death certificate)

Data from 29 well-preserved white Caucasian cadavers of subjects aged 65 years and over (17 female and 12 male) are reported here. Out of one BCAS project 14 female and 9 male cadavers were included, with a mean age of $77,5 \pm 6,9$ years (Clarys et al., 1984, 1999). Data from three male and three female additional cadavers with a mean age of $80,7 \pm 6,8$ years were obtained from another BCAS dissection project (Clarys et al., 1999; Janssens et al., 1994). All cadavers were embalmed within 48 hours after death. All applicable institutional, governmental and legal regulations concerning the ethical approval of human volunteers were followed during the study.

The BCAS projects provided anthropometric measures allowing for the calculation of BMI and WC. Supine length was measured with the cadaver on a horizontal surface, using a custom-made anthropometer. Body mass index was calculated as weight divided by height squared (kg/m^2). For ease of measurement, the cadaver was suspended by an adapted orthopaedic head harness, and manipulated by a pulley attached to the ceiling. Waist circumference (the smallest girth between the iliac crest and the costal border) was measured with a flexible plastic tape ruler to the nearest 0.1 cm. All measurements were performed with the cadaver warmed to ambient temperature (24°C) in order to limit temperature-related differences in texture and mobility of the skin and AT.

The cadavers were weighed immediately before dissection, which started in the early morning and continued until completion ($\pm 14\text{-}20\text{h}$ later). All cadavers were dissected into their various components expressed on the tissue-system level i.e. skin, muscle, adipose tissue, viscera and bones; which were weighed to the nearest 0.001kg with dehydration reduced to a minimum (Wang et al., 1992). Detailed methodology of dissection procedures has been reported elsewhere (Clarys et al., 1984, 1999; Martin et al., 2003a). The evaporative loss of body fluid during the dissection was calculated as the difference between total body weight before dissection and total tissue weight after dissection. The individual loss in each cadaver was allocated back to the different tissues in proportion to their respective masses. After this correction, the sum of the weights of all dissected tissues was equal to the cadaver's whole body weight prior to dissection.

Six body segments were defined: the four limbs, trunk and head. Weights of all tissues were recorded as total body adipose tissue mass (AT), trunk subcutaneous adipose tissue mass

(SAT) and trunk internal adipose tissue mass (IAT, the sum of intra-abdominal AT (i.e. visceral and retroperitoneal AT) and intra-thoracic AT), muscle tissue mass, bone tissue mass, skin tissue mass and visceral tissue mass.

Three measures of muscle to adipose tissue proportions were considered: the ratio of muscle mass to AT, the ratio of muscle mass to IAT and the ratio of muscle mass to SAT. Two measures of regional trunk adipose tissue proportion were calculated also: the ratio of IAT to AT and the ratio of IAT to SAT.

Statistical Package for Social Sciences (version 17.0.1 for Windows, SPSS Inc, Chicago, USA) was used for the data analysis. Data are reported as mean \pm standard deviation. Normality of data distributions was verified using Kolmogorov-Smirnov Goodness of Fit test ($p > 0.05$). Gender differences in BC were calculated using unpaired t-tests. The relationships of BMI and WC with BC constituents were assessed using Pearson correlation coefficients. Subjects were classified according to BMI based on the International Classification of adult underweight (BMI ≤ 18.5 kg/m²), overweight (BMI ≥ 25 kg/m²) and obesity (BMI ≥ 30 kg/m²) as defined by the WHO (2000). Females and males were also classified in low-risk (females, < 80 cm; males, < 94 cm), moderate-risk (females, ranging from 80 cm to 88 cm; males, ranging from 94 cm to 102 cm) and high-risk (females, ≥ 88 cm; males, ≥ 102 cm) WC categories as proposed by Lean and colleagues (1995).

3.2 Direct relationship of body mass index with body tissue distribution

Total body weight (BW) for the whole sample before dissection was 60.0 ± 12.9 kg. Evaporative loss of fluid (ELF) during dissection was 2.0 ± 0.6 kg and the accuracy of the whole-body dissection method (ELF/BW) ranged from 0.6% to 6.8% (mean = $3.3 \pm 1.3\%$).

Compared to female, male were significantly taller ($p < 0.01$) and showed lower AT ($p < 0.05$), higher muscle ($p < 0.01$), bone ($p < 0.001$) and visceral tissue masses ($p < 0.05$); higher muscle to AT ratio and muscle to SAT ratio ($p < 0.01$), and higher proportions of IAT ($p < 0.01$) (Table 7). No significant gender differences were found for age, weight, BMI and WC.

BMI and WC were significantly and positively related to various tissue masses in both sexes (Table 8). Muscle tissue, AT and IAT correlated better with BMI (r-values between 0.68 and 0.89) than with WC (r-values between 0.49 and 0.71). SAT correlated equally well with BMI (r-values between 0.61 and 0.78) and with WC (r-values between 0.62 and 0.83).

Both in females and in males BMI was significantly and inversely related with ratios of muscle mass to AT masses (Table 8). Both muscle tissue mass and AT masses increase with BMI in a quasi-linear manner; their ratio, however, decreases with increasing BMI. Visual inspection of BC graphs revealed major differences in muscle tissue mass proportions according to gender and WHO cutoff-values for BMI. For example, the ratio of muscle mass to AT mass ranged from 0.5 to 2.5 in males with normal BMI-values, and the ratio of muscle mass to IAT mass was not significantly different in subjects with normal BMI compared to those presenting an elevated BMI (Figure 3).

Waist circumference correlated significantly and inversely with ratios of muscle mass to AT masses in females, but not in males (Table 8). Visual inspection of BC graphs revealed important differences in muscle tissue mass proportions based on WC categories, similar to those found based on BMI-classification. This is not surprising given the fact that BMI and WC are highly correlated both in females ($r = 0.77$; $p < 0.001$) and in males ($r = 0.91$; $p < 0.001$).

| | Female (n = 17) Mean ± sd (range) | Male (n = 12) Mean ± sd (range) |
|---------------------------------|--------------------------------------|---------------------------------------|
| <i>Physical characteristics</i> | | |
| Age (years) | 79,9 ± 7,1 (68-94) | 75,6 ± 6,1 (65-87) |
| Weight (kg) | 58,8 ± 11,6 (32,0-75,4) | 61,7 ± 14,9 (38,5-85,7) |
| Height (m) | 1,59 ± 0,07 (1,46-1,73) | 1,67 ± 0,06 (1,60-1,80) [†] |
| BMI (kg/m ²) | 23,4 ± 4,6 (12,9-30,9) | 21,9 ± 4,3 (14,7-28,4) |
| Underweight (n) | 2 | 2 |
| Normal weight (n) | 9 | 7 |
| Overweight (n) | 4 | 4 |
| Obese (n) | 1 | 0 |
| WC (cm) | 80,4 ± 7,3 (69,7-94,0) | 83,4 ± 7,0 (73,1-94,3) |
| Low-risk (n) | 9 | 10 |
| Moderate-risk (n) | 6 | 1 |
| High-risk (n) | 2 | 1 |
| <i>Body composition</i> | | |
| Total body adipose tissue (kg) | 23,2± 8,9 (4,6-40,1) | 16,4 ± 6,8 (5,7-25,7) [*] |
| Trunk Subcutaneous AT (kg) | 7,6 ± 3,1 (2,5-13,4) | 5,4 ± 2,7 (2,6-10,4) |
| Trunk Internal AT (kg) | 3,1 ± 1,7 (0,3-5,8) | 3,0 ± 1,6 (0,5-5,3) |
| Skin (kg) | 3,2 ± 0,6 (1,7-4,1) | 3,5 ± 0,7 (2,5-4,7) |
| Muscle (kg) | 17,1 ± 3,2 (12,2-23,4) | 22,5 ± 6,2 (14,0-34,8) [†] |
| Bone (kg) | 7,7 ± 0,8 (6,7-10,0) | 9,6 ± 1,5 (7,4-12,6) [‡] |
| Viscera (kg) | 7,5 ± 1,4 (5,8-10,7) | 9,8 ± 3,2 (6,3-18,9) [*] |
| Muscle/AT | 0,90 ± 0,53 (0,36-2,70) | 1,56 ± 0,57 (0,65-2,46) [†] |
| Muscle/IAT | 9,4 ± 10,8 (2,4-46,2) | 10,7 ± 7,7 (3,1-26,9) |
| Muscle/SAT | 2,7 ± 1,2 (1,0-5,0) | 4,9 ± 1,8 (2,0-9,1) [†] |
| IAT/AT (%) | 12,6 ± 3,5 (5,3-17,6) | 17,6 ± 5,3 (9,1-24,8) [†] |
| IAT/SAT (%) | 40,5 ± 15,5 (10,9-73,9) | 58,9 ± 27,9 (18,8-116,9) [*] |

Table 7. Physical Characteristics and Body Composition of the Subjects (sd = standard deviation, BMI=body mass index, n=total number of subjects, WC=waist circumference, AT=total body adipose tissue, IAT=trunk internal adipose tissue, SAT=trunk subcutaneous adipose tissue. ^{*}*p*<0,05, [†]*p*<0,01, [‡]*p*<0,001)

| | BMI | | WC | |
|------------|--------|--------|--------|-------|
| | Female | Male | Female | Male |
| Muscle | 0.68† | 0.89‡ | 0.50* | 0.71† |
| AT | 0.80‡ | 0.84† | 0.67† | 0.70* |
| IAT | 0.72† | 0.68* | 0.49* | 0.44 |
| SAT | 0.61† | 0.78† | 0.62† | 0.83† |
| Muscle/AT | -0.67† | -0.62* | -0.64† | -0.49 |
| Muscle/IAT | -0.63† | -0.68* | -0.55* | -0.36 |
| Muscle/SAT | -0.54* | -0.42 | -0.55* | -0.57 |
| IAT/AT | 0.54* | 0.40 | 0.23 | 0.07 |
| IAT/SAT | 0.50* | 0.18 | 0.16 | -0.09 |

Table 8. Pearson correlation coefficients for the relationships of BMI and WC with BC in 17 female and 12 male cadavers by dissection (BMI=body mass index, WC=waist circumference, AT=total body adipose tissue, IAT=trunk internal AT, SAT=trunk subcutaneous AT. * $p<0,05$,† $p<0,01$,‡ $p<0,001$)

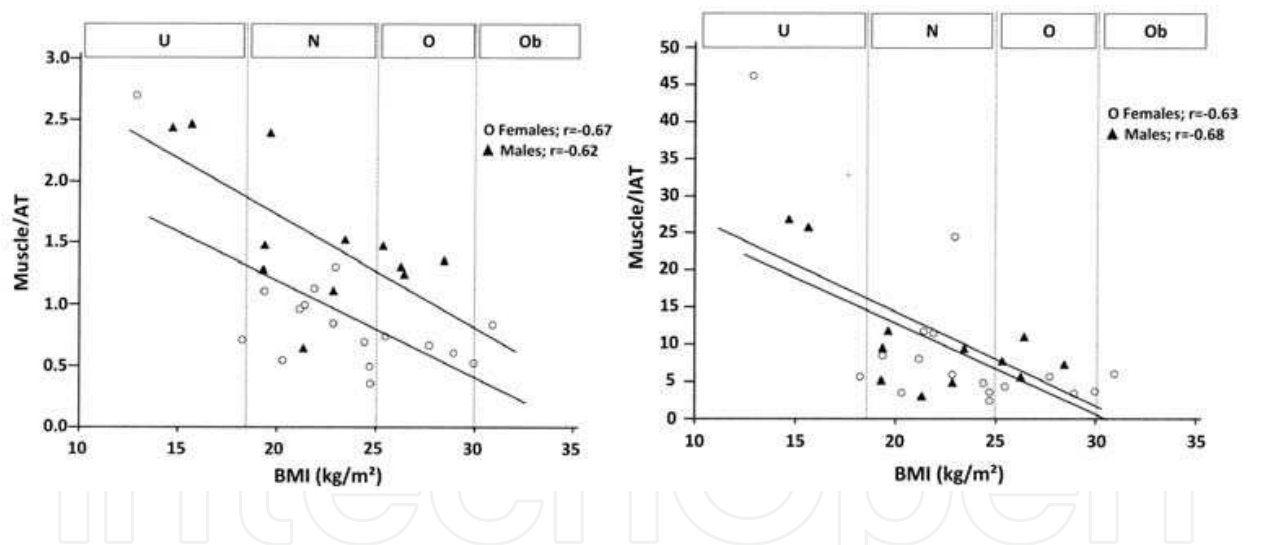


Fig. 3. Relationship of BMI with muscle tissue mass proportions in 29 elderly cadavers (U=underweight, BMI<18,5; N=normal weight, 18,5≤BMI<25; O=overweight, 25≤BMI<30; Ob=obese, BMI≥30)

Body mass index correlated significantly to measures of trunk adipose tissue proportions in females, but not in males ($p<0.05$; Table 3). Waist circumference was not significantly related to the ratio of IAT to AT nor to the ratio of IAT to SAT in our sample ($p>0.05$; see Table 3). Visual inspection of the graphs shows that trunk AT distribution varies considerably between sexes and within categories. For example, the ratio of IAT to SAT was not different between low-risk and moderate-risk females (Figure 4).

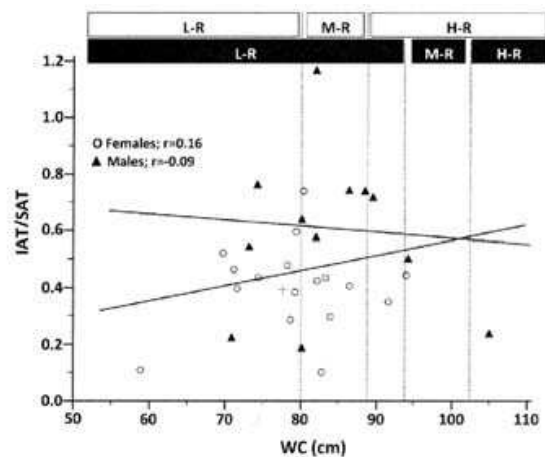


Fig. 4. Relationship of WC with trunk adipose tissue distribution in 29 elderly cadavers (□ = Female categories: L-R=low-risk, WC<80cm; M-R=moderate-risk, 80cm≤WC<88cm; H-R=high-risk, WC≥88cm; ■ = Male categories: L-R=low-risk, WC<94cm; M-R=moderate-risk, 94cm≤WC<102cm; H-R=high-risk, WC≥102cm).

3.3 Inter-individual and sex specific differences in body composition

Understanding the relationship between BMI, WC and BC in the elderly may provide better interpretation of these measures in clinical practice (Bedogni et al., 2001). The exact determination of the muscle and adipose tissue compartments is difficult in living humans, and mainly based on ‘reference’ BC methods such as CT or MRI (Abate et al., 1994; Mitsiopoulos et al., 1998). It needs consideration that this is the first report relating BMI and WC to directly obtained measurements of the muscle and adipose tissue compartments in elderly subjects (Martin et al., 2003a; Scafoglieri et al., 2010).

The present design is unique in the sense that it requires no assumptions regarding the measurement and the calculation of the BC constituents. It shows that moderate to strong relationships of BMI and WC with absolute tissue masses and with muscle tissue mass proportions in elderly subjects exist. These results confirm the findings of previous validation work using CT and MRI on living subjects (Ferrannini et al., 2008; Kvist et al., 1988; Lee et al., 2000; Ludesher et al., 2009). However cautious clinical interpretation is warranted since important inter-individual differences in tissue proportions were found in subjects with similar BMI and/or WC values.

Sarcopenic-obesity has been defined as a condition in elderly persons reflected by low muscle mass (sarcopenia) in combination with high AT mass (obesity) (Zamboni et al., 2008). Although it is unclear which clinical condition, sarcopenia or obesity, may precede in the development of sarcopenic-obesity, it is suggested that the age-related increase in adipose tissue mass generally precedes the loss of skeletal muscle mass (Rolland et al., 2009). The BMI and WC may offer the clinician a practical anthropometric measurement for assessing a subject’s whole body and visceral AT content. In our sample sex specific differences in BC were found, elderly females proportionally having more adipose tissue than males of similar age and BMI, who in turn are more muscular. Consequently the ratio of muscle mass to total body AT mass was found to be significantly higher in males compared to females. The observation that BMI is significantly and inversely related to the ratio of muscle to total body AT mass for both sexes in the present study, might validate the association of BMI with the lean/fat ratio as determined by BIA (Ozenoglu et al., 2009). It

has to be pointed out that the significant inverse relationship between BMI and the measures of muscle mass distribution in this sample may result from the high muscle tissue proportions of the individuals classified as underweight. It has been suggested previously that regional muscle/AT ratio is closely related to aging and to visceral AT accumulation (Kitajima et al., 2010). Interestingly and in contrast to the sex specific differences in total body adiposity and muscularity, internal AT mass was not different between females and males in our sample. Since the latter represents a major metabolic compartment within the body, this observation might be of great importance. Although BMI is related to IAT in the present study, it has to be pointed out that important inter-individual differences within and between adjacent WHO-classifications do exist. Elderly individuals with similar BMI-values do not necessarily present similar levels of internal adiposity. This observation might jeopardize the clinical interpretation of the association between BMI and BC compartments based on BMI alone. These results suggest that additional assessment (such as imaging methods) may be indicated in order to quantify this important metabolic compartment. In this context, it has been suggested that ultrasound is able to account for visceral adiposity although this may be debatable (Martin et al., 2003b).

Besides the determination of absolute AT quantities, its distribution within the body is an important health consideration (Baumgartner et al., 1995). It is well known that visceral AT concentration carries greater cardiovascular health risk compared to subcutaneous AT accumulation (Larsson et al., 1992). Visceral AT and subcutaneous AT can predict different health-risks, based on their own morphological and functional features, even for a given level of abdominal adiposity (Sniderman et al., 2007). Visceral AT has been repeatedly linked to an increased risk of dyslipidemia, dysglycemia and vascular disease. By contrast, subcutaneous AT has been associated with better metabolic outcomes. This study observed sex specific differences in trunk adipose tissue distribution. Elderly males showed lower AT mass but higher proportions of internal AT compared to females of similar age and similar BMI. This observation supports previous findings as determined by MRI (Ferrannini et al., 2008). In our sample BMI was positively related to regional AT distribution in females only, suggesting that BMI-values do not allow distinction between internal and subcutaneous AT accumulation in elderly males. This is partly in agreement with the findings of Seidell et al. (1987) who found no significant correlations between BMI and the ratio of visceral to subcutaneous AT area using CT in a younger population (Seidell et al., 1987). Waist circumference is generally accepted as a practical measurement for assessing a subjects visceral AT content. However, since WC is a composite measure of visceral and subcutaneous AT, it might not distinguish visceral from subcutaneous AT. To our knowledge, no recent studies are available reporting the relationship of WC with trunk AT distribution (as defined in this chapter). In the present study, WC was not significantly correlated to measures of trunk AT distribution, such as the ratio of IAT to SAT. It should also be observed that WC was a better correlate of SAT than of IAT in both sexes, suggesting that WC might be a more appropriate indicator of subcutaneous than of internal adiposity, in particular in elderly males. This observation supports previous findings using MRI in vivo (Ferrannini et al., 2008). These results indicate that inter-individual differences in trunk adipose tissue composition might not be detected by simple anthropometric measures such as BMI or WC, in particular in elderly persons.

3.4 Limitations of post mortem cadaver dissections

The 'reference' method for the determination of BC presented here was cadaver dissection. Although this method has limitations including tissue dehydration, an age matched in vivo

and post mortem constitutional and anthropometric comparison has shown an overall similarity of macroscopic characteristics between subjects (Clarys et al., 2006). Since no data are available on the duration of the clinical-pathologic status of the subjects, it remains unclear to which extent body composition might have been affected in the chronically ill subjects (n=6). On the other hand, it has to be pointed out that adiposity indices such as BMI and WC are regularly used in the evaluation and follow-up of the nutritional status both in healthy elderly and in patients. The precision of our method to determine BC averaged 3,3%, which indicates that dehydration and/or losses of material during the dissection procedures were negligible. It is therefore unlikely that the method of choice biased the results presented here. Moreover the mean difference between actual weight and CT derived or MRI estimated weight reaches 5,6% to 6,0%, the latter being considered as a gold standard method in BC (Baumgartner et al., 1995; Clarys et al., 1999). An inevitable restriction proper to a whole-body dissection is the relatively limited number of individuals whose BC can be determined. This is due to the work-related intensity of the dissection procedures combined with the limited availability of subjects. Results of the nature as presented here should preferably be confirmed in a larger sample, but one must realize that such opportunities and possibilities will remain very cumbersome, difficult and scarce.

3.5 Critical appraisal of the Body Mass Index as a body composition tool

This post mortem *in vitro* evaluation suggests that BMI and WC are significantly related with adipose tissue mass and with several ratio's of muscle to adipose tissue in elderly subjects. However elderly persons with similar BMI and/or WC values do not necessarily present similar tissue mass proportions, limiting their use when comparing individual BC within and between adjacent classification systems. Since BMI and WC are composite measures of BC, assessment of important metabolic body compartments themselves is warranted in elderly persons (Scafoglieri et al., 2010).

4. Dual energy X-ray absorptiometry: What are we measuring?

Although BC data acquisition and ad hoc 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 the fifties and sixties, with the pioneer work of Keys & Brozek (1953), Forbes et al. (1956), Siri (1956), Brozek et al. (1963), Behnke (1963), Durnin & Rahaman (1967), body composition almost became a scientific discipline profiling itself with the development of many methods, techniques and equipment. 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 HD (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; Martin et al., 1985, 1992) and the universally accepted new method of choice, the dual energy X-ray absorptiometry or DXA (Bolotin, 1998, 2007; Bolotin & Sievanen, 2001; Bolotin et al., 2001; Clarys et al., 2010b; Proven et al., 2008).

4.1 Validation of dual energy X-ray absorptiometry

Curiously, after reviewing the literature of DXA application, one cannot avoid obtaining a very controversial impression of this new method. On the other hand, we find an important

number of validation and application studies that support the DXA technique as convenient, as the criterion for %fat, for lean body mass (LBM), and as a criterion for 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). In addition to the violation of basic assumptions as referred to earlier, one needs to repeat and underline that DXA, hydrodensitometry, anthropometry, air-, gas- and water displacement methods, bioelectrical impedance (BIA) are all indirect *in vivo* techniques 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; Provyn et al., 2008). 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) and with the basic confusion between overall BC terminology e.g. fat, adipose tissue (AT), fat free mass (FFM), LBM, lean, adipose tissue free mass (ATFM), bone mineral density (BMD), surface and volume density, bone mineral content (BMC), ash weight, actual mineral content and BMC, with or without soft tissue covering (Clarys et al., 2010b; Martin et al., 1985; Provyn et al., 2008; 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 body composition 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). A review of the state of the art of carcass studies related to DXA (Clarys et al., 2008) 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 hind legs (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. Almost all studies indicated perfect correlations for all variables with DXA, but approximately half of the results of the various variables were found to be significantly different ($p < 0.001$ and $p < 0.05$). In approximately a third of these studies, DXA was suggested to be valid and accurate for all its variables, while 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: a) dissection and direct comparison combined with bone ashing is considered the most accurate and direct validation technique (Elowsson et al., 1998) and b) 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). Since BC measurements by DXA are increasingly used in clinical practice and because dissection is the best possible direct measure, no study

has been giving clarity yet about the content and meaning of “lean” as produced by DXA, different intra-tissue combinations, e.g., skin, muscle, viscera and bone will be related to the DXA-lean variable. Exact knowledge of what is the content of the meaning of “lean” as measured by DXA is mandatory. In this chapter section we will compare DXA fan beam data, with both dissection and CT scanning data.

4.2 Methodology

Twelve, 6-18 month-old “Belgian Native” pigs were prepared for human consumption and were acquired within 2 days intervals, immediately after electroshock slaughter (6 female and 6 castrated males, mean weight \pm standard deviation (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 non-removal 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 hind legs were disarticulated distal from humeri and femora e.g., on elbow and knee level, respectively. The mean weight \pm sd 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 50g. The composition of the carcasses was studied in the following order.

A QDR 4500A upgraded to Discovery HOLOGIC DXA device (Hologic, Waltham, MA, USA) utilizes a constant X-ray source producing fan beam dual energy radiation with effective dose equivalents (EDE) of $5 \mu\text{Sv}$ (Prentice, 1995).

The estimations of fat and lean mass are based on extrapolation of the ratio of soft tissue attenuation of two X-ray energies in non-bone-containing pixels. The two X-ray energies are produced by a tungsten stationary anode X-ray tube pulsed alternately as 70 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 for each carcass of percentage of fat, fat mass (g), lean mass (g), bone mineral mass (g), BMD in g/cm^2 and total weight. According to the manufacturer, a coefficient of variation (CV) for human BMD of 0.5% can be expected during repeated measurements.

To determine the reliability of DXA measurements, each pig carcass was scanned three times consecutively without (2x) and with (1x) repositioning. From these data, the CV for the different tissue types was calculated.

The 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.

Whole body scans of the pigs were taken with a CT scanner (type Philips Brilliance BZC 16, Koninklijke Philips Electronics NV, Eindhoven, The Netherlands) using the following settings: 120 kVp, 200 mAs, pitch 0.641, slice collimation 64×0.625 mm, reconstructed slice width 0.75 mm and using the Brilliance™ V2.3.0.16060 software. Tissues (Adipose tissue = AT, soft tissue = ST and bone = B) were classified based on Hounsfield Units (HU) and their respective volumes were calculated using a maximum likelihood Gaussian mixture estimator implemented in Matlab (The Mathworks Inc., Natick, United States). The following optimal classification scale was employed to determine each tissue: AT: -180..-7 HU; ST: -6..+142 HU and B: +143..+3010 HU (McEvoy et al., 2008; Vester-Christensen et al., 2009). Tissue volumes were multiplied by their reference densities with AT= $0.923 \text{ g}/\text{cm}^3$, ST= $1.040 \text{ g}/\text{cm}^3$ and B= $1.720 \text{ g}/\text{cm}^3$ to obtain tissue weight estimates.

After the DXA measurements, the carcasses were dissected into their various components as expressed on the tissue-level system: skin, muscle, adipose tissue, viscera and bones (Wang et al., 1992). Muscle included tendon, blood vessels and nerves belonging to the ad hoc muscle. The subcutaneous, intramuscular (mostly intra-tendon) and intra-visceral AT was 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 pro-sectors and anatomists worked simultaneously and each dissected particle was collected under cling film and kept in color-labeled, continuously covered plastic containers (12x10x10 cm) of known weight in order to minimize or eliminate evaporation (Clarys et al., 1999, 2010b; Provyn et al., 2008). Full containers mass was measured during the dissection by 2 researchers using Mettler-Toledo digital scales (Excellence XS precision balance Model 40025) accurate to 0.01g. Once a bone was fully prepared, the same procedure was followed but completed with its hydrostatic weight whilst placed in a wire cradle suspended to the same scale allowing for the volume-based bone density (g/cm^3) calculation.

After the dissection and multiple weighing procedures, samples of all tissues of approximately 100g to 150g (min-max) were deep-frozen. Small parts were cut off and weighed in recipients of known weight before lyophilisation 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 twelve porcine carcasses was the total defleshing of the skeleton, including the removal of extra-osseous soft tendon and ligament tissue by scraping. Cartilage and intra-osseous tissue (e.g. intervertebral discs) remained intact. The whole skeleton was diamond-cut into pieces in order to fit in the ashing furnace (type Nabertherm, Liliental, Germany). After incineration, each sample was heated using a ramped temperature protocol of two hours to 800°C and ashed for eight hours, as determined by prior pilot work. Before weighing on the Mettler Toledo precision scale (accurate to 0.01g) the ash was cooled undercover and collected in a main container. The ashing of one full porcine skeleton took between 50 to 60 hours.

Data are reported as mean(x) \pm standard deviation(sd). Normality of all variables was verified with a Kolmogorov-Smirnov test and all DXA, CT and dissection data were (matrix) compared with Pearson correlation coefficients, while differences were verified with one-way analysis of variance repeated measures (Anova). Reliability and consistency of these results were verified with intra-class correlations (ICC) and Bland-Altman plots were used to access agreement of the direct carcass dissection data with the indirect DXA and CT estimates. All statistical tests were performed using SPSS 16.0 for windows and p values of <0.05 indicated significant differences.

4.3 Definition, quantification and comparison of DXA variables

Comparing directly and indirectly obtained data of masses and densities (e.g. of whole body bone-, adipose- and non adipose tissue) using 3 different techniques yields information on the ad hoc terminology used in the respective methodologies. Table 9 shows an overview of terminology used per technique as applied and the assumed measure of the same values.

| Dissection | DXA | CT | Biological background |
|---------------------------------------|--|-----------------------------------|--|
| Total mass (g) | Total mass (g) | Total mass (g) | - |
| Total tissue mass (g) | Total mass (g) | Total mass (g) | The Σ of all dissected tissue masses |
| Adipose tissue (g) | Fat (g) | Adipose tissue(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) | Fat free mass (FFM) (g) | ATFM is an anatomical concept LBM = FFM plus essential lipids |
| Skeleton mass (g) | Bone mineral content (BMC)(g) | Bone mass (g) | Skeleton and bone mass are morphological issues; BMC suggests the Σ of all mineral constituents of the skeleton |
| Skeleton density (g/cm ³) | Bone mineral density(g/cm ²) | Bone density (g/cm ³) | Volume (g/cm ³) based versus surface (g/cm ²) based density |

Table 9. Different terminologies assumed to measure a similar outcome (DXA=dual energy X-ray absorptiometry, CT=computed tomography)

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 10). Table 10 combines the data acquisition of all directly obtained measures and the complete set of indirect estimates made by DXA and CT. The purpose of this Table 10 is to evaluate the predictive quality of both DXA and CT, 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 $r \geq 0.70$ an average (mediocre) indicator of prediction confirmed or rejected by the ICC. The Anova 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. A non-significant result between DXA and CT indicates similarity between data only, since DXA nor CT is considered to be a reference in this study.

Table 10 confirms that for almost all soft tissue comparisons, including total masses, a majority of good correlations ($r \geq 0.90$), two medium correlations ($r \geq 0.80$) and two average ($r \geq 0.70$), adiposity prediction expressed in % seems to be problematic for the CT. Despite the majority of good prognoses for prediction related to the dissection reference, we do find significant differences in accuracy for total masses (DXA), adiposity (g and %)(DXA and CT) for all non-adipose soft tissue combinations (DXA and CT) and for all bony comparisons. Except for the ashing, there are indications of acceptable precision and comparability with DXA-BMC. The ICC and the Bland-Altman plots confirm the findings as shown in Table 10.

| Variables | Dissection x ± sd | DXA x ± sd | CT x ± sd | r | Anova F | ICC |
|-----------------------------------|----------------------|------------------|------------------|------|-----------|-------|
| Total mass(g) | 33051.3 ± 3323.8 | 33192.3 ± 3336.6 | -- | 1.00 | 17.903† | 1.00‡ |
| | 33051.3 ± 3323.8 | -- | 33041.7 ± 3337.8 | 0.99 | 0.006 | 0.99‡ |
| | -- | 33192.3 ± 3336.6 | 33041.7 ± 3337.8 | 0.99 | 1.463 | 0.99‡ |
| Total tissue mass(g) | 32723.4 ± 3427.0 | 33192.3 ± 3336.6 | -- | 1.00 | 24.061‡ | 0.99‡ |
| | 32723.4 ± 3427.0 | -- | 33041.7 ± 3337.8 | 0.98 | 2.689 | 0.98‡ |
| Adipose tissue/Fat(g) | 3571.6 ± 632.8 | 5653.1 ± 934.1 | -- | 0.91 | 268.516‡ | 0.85‡ |
| Adipose tissue/ Adipose tissue(g) | 3571.6 ± 632.8 | -- | 5508.3 ± 844.7 | 0.72 | 131.446‡ | 0.69† |
| Fat/ Adipose tissue(g) | -- | 5653.1 ± 934.1 | 5508.3 ± 844.7 | 0.80 | 0.777 | 0.80† |
| Adipose tissue/Fat (%) | 10.8 ± 1.27 | 17.0 ± 1.87 | -- | 0.81 | 370.409‡ | 0.76† |
| Adipose tissue/ Adipose tissue(%) | 10.8 ± 1.27 | -- | 16.6 ± 1.19 | 0.31 | 195.514‡ | 0.31 |
| Fat/ Adipose tissue(%) | -- | 17.0 ± 1.87 | 16.6 ± 1.19 | 0.46 | 0.594 | 0.41 |
| ATFM/Lean+BMC(g) | 29479.7 ± 2874.7 | 27544.7 ± 2681.5 | -- | 0.99 | 227.140‡ | 0.99‡ |
| ATFM/Soft Tissue+Bone(g) | 29479.7 ± 2874.7 | -- | 27525.0 ± 2559.9 | 0.98 | 142.665‡ | 0.98‡ |
| Lean+BMC/Soft Tissue+Bone(g) | -- | 27544.7 ± 2681.5 | 27525.0 ± 2559.9 | 0.97 | 0.012 | 0.97‡ |
| Muscle/Lean(g) | 17684.3 ± 1908.8 | 27103.1 ± 2647.3 | -- | 0.95 | 1012.029‡ | 0.90‡ |
| Muscle/Soft Tissue(g) | 17684.3 ± 1908.8 | -- | 24166.7 ± 2270.1 | 0.94 | 790.922‡ | 0.93‡ |
| Lean/Soft Tissue(g) | -- | 27103.1 ± 2647.3 | 24166.7 ± 2270.1 | 0.97 | 196.183‡ | 0.96‡ |
| Skin | 1326.7 ± 244.0 | -- | -- | -- | -- | -- |
| Muscle+skin/Lean(g) | 19011.1 ± 2092.3 | 27103.1 ± 2647.3 | -- | 0.95 | 960.440‡ | 0.93‡ |
| Muscle+skin/Soft Tissue(g) | 19011.1 ± 2092.3 | -- | 24166.7 ± 2270.1 | 0.95 | 642.421‡ | 0.95‡ |
| Viscera | 7465.3 ± 803.8 | -- | -- | -- | -- | -- |

| Variables | Dissection x ± sd | DXA x ± sd | CT x ± sd | r | Anova F | ICC |
|--------------------------------------|----------------------|------------------|------------------|------|----------|-------|
| Muscle+skin+viscera/Lean(g) | 26476.4 ± 2593.8 | 27103.1 ± 2647.3 | -- | 0.99 | 61.326‡ | 0.99‡ |
| Muscle+ skin+viscera/Soft Tissue(g) | 26476.4 ± 2593.8 | -- | 24166.7 ± 2270.1 | 0.97 | 162.206‡ | 0.97‡ |
| Skeleton mass/BMC(g) | 2505.3 ± 317.5 | 441.6 ± 64.6 | -- | 0.62 | 641.302‡ | 0.24 |
| Skeleton mass/Bone mass(g) | 2505.3 ± 317.5 | -- | 3358.3 ± 446.1 | 0.59 | 65.404‡ | 0.55* |
| BMC/Bone mass(g) | -- | 441.6 ± 64.6 | 3358.3 ± 446.1 | 0.40 | 566.598‡ | 0.11 |
| Ash weight/BMC(g) | 445.6 ± 66.2 | 441.6 ± 64.6 | -- | 0.73 | 0.086 | 0.73‡ |
| Skeleton Density(g/cm³)/BMD(g/cm²) | 1.201 ± 0.02 | 0.782 ± 0.09 | -- | 0.68 | 370.144‡ | 0.24 |
| Skeleton Density/Bone density(g/cm³) | 1.201 ± 0.02 | -- | 1.720 ± ND | ND | ND | ND |
| BMD(g/cm²)/Bone density(g/cm³) | -- | 0.782 ± 0.09 | 1.720 ± ND | ND | ND | ND |

Table 10. Comparison between direct dissection data values with the corresponding DXA and CT values (DXA=dual energy X-ray absorptiometry, CT=computed tomography, x=mean, sd=standard deviation, r=Pearson correlation coefficient, ICC=intra-class correlation coefficient, ATFM=adipose tissue free mass, BMC=bone mineral content, **p*<0.05, †*p*<0.01, ‡*p*<0.001, ND=not determined, CT considers bone density as a constant value)

4.4 Variation of hydration status and lipid content of tissues

The dissection tissue masses were subdivided according to anatomic segmentation into upper limb, lower limb and trunk (e.g. for skin, muscle and bone). For adipose tissue, 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 11.

Body fat (BF) is defined as the ether-extractable constituent of body tissues, (Table 11) and must be considered as a chemical component of the body. This is already known since Keys & Brozek (1953). The interchangeable use of the terms BF and AT has led and is leading still to ambiguities and serious error. Amongst 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). Table 1 indicates other discrepancies e.g., for the non-adipose terminology. Adipose tissue free mass is an anatomical concept and lays in the continuation of the AT versus FM. DXA pretends to measure Lean or Lean Body Mass as opposed to FFM, which could be expected since manufacturers claim to measure chemical components.

If we look at the mean value level of the respective variables in Table 10, there cannot be any doubt that both DXA and CT are producing anatomical-morphological quantities, evidently at all adipose and non-adipose combinations. In addition DXA and CT do not take into

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

Table 11. Water (lyophilisation) and lipid (ether extraction) content of different tissues and relationship (x=mean, sd=standard deviation, r=Pearson correlation coefficient, †p<0.01)

account the water content and lipid content variations (Table 11) of both its adipose and non adipose constituents. Small variation of tissue hydration may explain important differences of ad hoc estimates (Prior et al., 1997; Wang et al., 1999, 1995).

Both in CT, 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. 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 (TWC) obtained by lyophylisation in several human tissue studies one can make two observations: 1) assuming a constant % of water in FFM may be jeopardized by the variable TWC within and between the tissues that compose FFM; and 2) water content in AT is highly variable e.g. ranging from ±17% to ±84% in humans (Provyn et al., 2008; Clarys et al., 2010a).

This is confirmed in our study on animal corpses with % whole body water content ranging from ±20 to ±50% (Table 11) repeating that the constancies claimed by DXA and CT cannot be maintained (e.g. with fluid ranging between ±50 to ±61% for skin, between ±39 to ±49% for bone but little variability for muscle).

Since 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. Sample masses being identical for hydration and lipid fractionation (Table 11) one learns 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 non systematically 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 hind legs 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 11 within a 4C 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 or LBM or ATFM reflects physiologic regulatory mechanisms (Going et al., 1993; Wang et al., 2005) cannot be answered, but it seems that trunk non-adipose tissues may affect hydration differently than the lean tissues of the extremities or vice-versa (Table 11).

4.5 Critical appraisal of DXA variables

Regardless of the existing mechanisms and regardless of the hydration and lipid (fat) content of non-adipose tissue, this macro quality evaluation has not been able to detect what the content is of the DXA non-adipose 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 and CT) resulted in equally high correlations (r -values between 0.94 and 0.99) assuming a good prediction estimate but with systematic significant difference confirming its imprecision "lean + 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. Contrarily 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 non-adipose values are expressed as anatomical-morphological values combined with chemical elements. We cannot confirm what the non-adipose component of DXA is measuring, but we do confirm that all the DXA components and the CT bone 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 hind legs 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 the present sample (Table 10), 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 hind leg 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 the present evaluation confirms that the DXA methodology cannot provide accurate, quantitative precise, meaningful determinations of true bone densities and proper bone mass because of the contamination of independent soft tissue, e.g., fluid and lipid content contributions.

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. This is confirmed. In terms of true “reality precision” measures, DXA produces inaccurate and misleading values at all levels of output. Both the adipose and non adipose components of DXA ignore the ad hoc lipid content and the non adipose 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. BMC versus ash weight is the only variable with a close reality and non significant difference output. DXA and CT are based on a series of constancies within tissues, regardless of segments, hydration and lipid content variability. The hypothesis that DXA methodology provides accurate, precise and relevant BC determinations are proven to be unwarranted and misplaced (Clarys et al., 2010b).

5. Conclusion

Accurate and precise measurement of human biological variation of tissue composition is both important and imperative in BC data acquisition. Together with the proliferation and abundance of different BC models, methods, techniques and equipment used in nutrition and health assessment, it is imperative that the BC data collector realizes that: a) all indirect models, techniques and devices are based upon assumptions and combined errors, b) different techniques for the same purpose may yield significant varying results... and c) at all times the assumption based prediction is a substantial different matter from accurate precision that is needed on the individual medical or other check-up. Within a clinical context, the borderline between prediction and accuracy has become vague and may need re-newed attention.

A closer collaboration between different scientific disciplines and stakeholders (nutritionists, clinicians, engineers and high technology companies) will contribute to increase the excellence of health-oriented BC research.

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