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From Methods to Applications: Molecular-Level Body Composition Assessment in Athletes

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“Essentially, all models are wrong, but some are useful.”

Box, George E. P. (1987). Empirical Model-Building and Response Surfaces. New York: Wiley, p. 424.

Abbreviations

%BF body fat percentage

2C two-compartment

$^{2}H_2O$ deuterium water

3C three-compartment

$^{3}H_2O$ tritiated water

$H_2{}^{18}O$ ^{18}O-labelled water

4C four-compartment

ADP air displacement plethysmography

ALST appendicular lean soft tissue

BD body density

BIA bioelectrical impedance analysis

BIS bioelectrical impedance spectroscopy

BMC bone mineral content

BMD bone mineral density

BF body fat

BV body volume

CT computed tomography

DXA Dual-energy X-ray absorptiometry

ECW extracellular water

FFM fat-free mass

FM fat-mass

ICW intracellular water

ISAK International Society for the Advancement of Kinanthropometry

LST lean soft tissue

MF-BIA multi-frequency bioelectrical impedance analysis

MRI magnetic resonance imaging

NCP Nutrition Care Process

PhA phase angle

R resistance

ROI regions of interest

SEM standard error of measurement

SF-BIA single-frequency bioelectrical impedance analysis

TBW total body water

TEM technical error of measurement

UWW underwater weighing

Xc reactance

Z impedance

Abstract

Accurate and precise body composition assessment is essential for nutrition and sports sciences. While multicompartment models, such as the four-compartment (4C) model, are considered the reference methods for fat mass (FM) assessment, it complexity, namely the use of dual-energy x-ray absorptiometry (DXA) to determine bone mineral content, deuterium dilution to assess total body water (TBW), and air displacement plethysmography (ADP) to measure body volume (BV), limits its practical application. Consequently, researchers and practitioners frequently rely on simpler two-compartment (2C) models or DXA. 2C models assume constant FFM density (1.100 g/cm^3) or hydration (≃73%); however, athletes often deviate from these assumed constants.

The present dissertation comprises four research articles in the field of Body Composition, with a general purpose of increasing knowledge about molecular-level body composition assessment in athletes. The primary emphasis was on rapid 4C models. These models replace the determination of BV from ADP with DXA-derived BV and/or the measurement of TBW using deuterium dilution with an estimate derived from bioelectrical impedance spectroscopy. Additionally, the precision of a less commonly examined step in the multi-step procedure for performing a whole-body DXA scan was investigated: the post-scan analysis.

The first study found that pre-existing DXA-derived BV equations showed significant differences compared to ADP when applied to athletes. Consequently, an equation (DXA-BV_{Silva}) was developed (n = 232) and validated (n = 100) in an athletic population. This new equation provided a robust alternative to ADP for estimating BV, with a negligible mean difference (0.05 L, SD = 0.46 L) and narrower 95% limits of agreement (-0.86 to 0.96 L) compared with those of existing equations.

The second investigation aimed to externally validate this equation (n = 143) and assess the accuracy of various rapid 4C models in athletes (n = 115). Fifteen alternative methods for estimating FM were evaluated in athletes from various sports. Among these, the rapid 4C model using ADP-derived BV and TBW derived from BIS (4C TBW_{BIS}) demonstrated the most accurate estimate of FM, with the narrowest 95% limits of agreement (-3.6% to 3.0%). The findings provided strong evidence for using a rapid 4C model in athletes when implementing the true criterion 4C model is impractical. It also indicated that rapid 4C models provide superior accuracy compared to traditional 2C methods and DXA (which exhibited wide limits of agreement of -8.6% to 4.4%).

Study 3 provided the first longitudinal evaluation of the validity of rapid 4C models for tracking changes in FM among athletes (n = 60). Rapid 4C models showed acceptable group-level agreement, but their wide 95% limits of agreement and systematic underestimation of changes raise concerns for individual-level monitoring. Even the rapid 4C model with the narrowest 95% limits of agreement, using ADP for BV and BIS for TBW, still exhibited a considerable range (-2.9% to 4.0%).

The fourth study investigated the reliability of DXA post-scan analysis between testers and the prevalence of inconsistencies between automatically defined regions of interest (ROIs) and manufacturer guidelines (n = 86). Despite nearly perfect intra- and inter-tester reliability (<1% mean difference; ICC = 0.998-1.000), study 4 found that manual review using a standardized protocol is essential. Inconsistencies between automatically defined ROIs and manufacturer guidelines were frequent, with chest lines (which separate the arm from the trunk) requiring corrections in 91.9% of scans.

Overall, this dissertation and the aforementioned studies make a substantial contribution to the literature on molecular-level body composition assessment in athletes, with the use of state-of-the-art methodologies representing a key strength of the work.

Key Words: athletes; body composition; dual-energy X-ray absorptiometry; multicompartment model; validation; reliability.

Resumo

A avaliação precisa e exata da composição corporal é essencial para as ciências da nutrição e do desporto. Embora os modelos multicompartimentais, como o modelo dos quatro compartimentos (4C), sejam considerados os métodos de referência para a avaliação da massa gorda (MG), a sua complexidade, nomeadamente a densitometria radiológica de dupla energia (DXA) para a determinação do conteúdo mineral ósseo, a diluição de deutério para a determinação da água corporal total (TBW) e a pletismografia por deslocamento de ar (ADP) para a determinação do volume corporal (BV), limita a sua aplicação prática. Consequentemente, investigadores e profissionais no terreno recorrem frequentemente a modelos mais simples, como o de dois compartimentos (2C) ou DXA. Os modelos dos 2C assumem uma densidade (1,100 g/cm³) ou uma hidratação (≃73%) da FFM constantes; contudo, os atletas frequentemente desviam-se destas constantes.

A presente tese compreende quatro artigos científicos na área da composição corporal e tem como objetivo geral ampliar o conhecimento sobre a avaliação da composição corporal ao nível molecular em atletas. A ênfase principal foi dada aos modelos 4C rápidos. Estes modelos substituem a determinação do BV por ADP pela estimativa por DXA e/ou a determinação da TBW por diluição de deutério pela estimativa por impedância bioelétrica de multifrequência espectral. Adicionalmente, foi estudada a precisão de uma etapa menos estudada do procedimento de avaliação por DXA: a análise pós-*scan*.

O primeiro estudo revelou que as equações existentes para estimar o BV a partir da DXA apresentavam diferenças significativas em relação à ADP quando aplicadas a atletas. Diante disso, foi desenvolvida (n = 232) e, posteriormente, validada (n = 100) uma equação (DXA-BV_{Silva}) numa população atlética. Esta nova equação revelou-se uma alternativa robusta à ADP para a estimativa do BV, com uma diferença média negligenciável (0,05 L, DP = 0,46 L) e limites de concordância a 95% mais estreitos (-0,86 a 0,96 L) quando comparada às equações existentes.

O segundo artigo teve como objetivo validar externamente a equação DXA-BV_{Silva} (n = 143) e avaliar a exatidão de vários modelos 4C rápidos em atletas (n = 115). Quinze métodos alternativos para estimar a MG foram avaliados em atletas de diversas modalidades. De entre estes, o modelo 4C rápido, recorrendo ao BV derivado da DXA e à TBW derivada da BIS (4C TBW_{BIS}), demonstrou a estimativa mais exata de MG e os limites de concordância de 95% mais estreitos (-3,6% a 3,0%). Os resultados forneceram evidência sólida para a utilização de um modelo

4C rápido em atletas quando a implementação do modelo 4C de referência é inviável. Demonstrou-se também que os modelos 4C rápidos apresentam exatidão superior à dos métodos 2C tradicionais e à DXA (que apresentou limites de concordância amplos de -8,6% a 4,4%).

O estudo 3 consistiu na primeira avaliação longitudinal da validade dos modelos 4C rápidos para monitorizar alterações da MG em atletas (n = 60). Os modelos 4C rápidos apresentaram resultados aceitáveis ao nível de grupo, mas os amplos limites de concordância a 95% e subestimação sistemática das alterações suscitam preocupações na monitorização da MG ao nível individual. O modelo 4C rápido com limites de concordância de 95% mais estreitos, recorrendo à ADP para o BV e à BIS para a TBW, apresentou uma amplitude considerável (-2.9% a 4.0%).

O quarto estudo investigou a fiabilidade da análise pós-scan da DXA entre avaliadores e a prevalência de inconsistências entre as regiões de interesse (ROIs) definidas automaticamente e as diretrizes do fabricante do equipamento (n = 86). Apesar de uma fiabilidade intra- e interavaliador quase perfeita (<1% de diferença média; ICC = 0.998-1.000), os resultados do estudo 4 revelaram que é essencial uma revisão manual com um protocolo padronizado. As inconsistências entre as ROIs definidas automaticamente e as diretrizes do fabricante foram frequentes, sendo necessárias correções das linhas de separação entre o braço e o tronco em 91,9% dos exames.

Coletivamente, esta dissertação e os estudos supracitados constituem um contributo substancial para a literatura sobre avaliação da composição corporal ao nível molecular em atletas, sendo o uso de metodologias de referência um ponto forte fundamental deste trabalho.

Palavras-chave: atletas; composição corporal; densitometria radiológica de dupla energia; modelo multicompartimental; validação; fiabilidade.

Chapter I - Dissertation structure

Dissertation structure

The present dissertation, entitled “From Methods to Applications: Molecular-Level Body Composition Assessment in Athletes”, comprises a compilation of four research articles: two are already published and two are submitted for publication in peer-reviewed journals with established ISI Impact Factors.

This dissertation is organized as follows:

This chapter (**Chapter I**) outlines the organization of the dissertation and provides a summary of the author’s scientific production during the doctoral program.

Chapter II comprises a general introduction to the topic, highlighting the importance of body composition assessment, elucidating its conceptual framework through hierarchical levels, describing the main models and measurement methodologies, and outlining the aims of this dissertation.

Chapter III briefly describes the methodologies employed across all studies and provides a comprehensive overview of the methods presented in each manuscript's methodology section.

Chapters IV to VII correspond to the studies conducted to address the research objectives outlined in Chapter II. Specifically, Chapter IV presents study 1: *Novel comparative analysis and equation development for body volume estimation using dual-energy x-ray absorptiometry in athletes*; Chapter V presents study 2: *Four-Compartment Models in Athletes Using Alternative Solutions for Body Volume and Water*; Chapter VI presents study 3: *Tracking Changes in Body Composition in Athletes: Are Rapid Four-Compartment Models Valid?*; and Chapter VII presents study 4: *Examining Intra- and Inter-Tester Reliability of Post-Scan Dual-Energy X-ray Absorptiometry Regional Body Composition Analysis in Athletes*.

Chapter VIII presents a general overview of the research and summarizes the main findings of each study, followed by a discussion of overall strengths and limitations. This chapter concludes by highlighting practical applications and suggesting directions for future research.

List of publications

Articles related to the dissertation (first author):

- **Silva, T. R.,** Jesus, F., Poínhos, R., Sardinha, L. B., Teixeira, V. H., Heymsfield, S. B., & Silva, A. M. (2025). Novel comparative analysis and equation development for body volume estimation using dual-energy x-ray absorptiometry in athletes. European Journal of Clinical Nutrition, 79(8), 740–747. https://doi.org/10.1038/s41430-025-01594-1
- **Silva, T. R**., Poínhos, R., Sardinha, L. B., Teixeira, V. H., & Silva, A. M. (2025). Rapid Four-Compartment Models in Athletes Using Alternative Solutions for Body Volume and Water. Scandinavian Journal of Medicine & science in Sports, 35(5), e70062. https://doi.org/10.1111/sms.70062
- **Silva, T. R.,** Poínhos, R., Oliveira, B. M. P. M., Santos, D. A., Matias, C. N., Sardinha, L. B., Teixeira, V. H., Heymsfield, S. B., & Silva, A. M. Tracking Changes in Body Composition in Athletes: Are Rapid Four-Compartment Models Valid? [Manuscript submitted for publication]
- **Silva, T. R.,** Gonçalves, B., Poínhos, R., Teixeira, V. H., & Silva, A. M. Examining Intra- and Inter-Tester Reliability of Post-Scan Dual-Energy X-ray Absorptiometry Regional Body Composition Analysis in Athletes. [Revisions submitted to the European Journal of Clinical Nutrition].

Other peer-reviewed articles (not related to the dissertation):

First author

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- Gonçalves, B. A., **Silva, T. R**., & Batista, A. Relationship between body composition and performance through a competitive season in rhythmic gymnastics [Manuscript submitted for publication].

Chapter II - Introduction

General introduction

The study of human body composition, spanning over 150 years, examines the masses and distributions of body components, their numerical relationships, *in vivo* quantification, and changes influenced by intrinsic and extrinsic factors[1]. Advances in this area have benefited researchers in academia, physicians, exercise physiologists and nutritionists in the field. This topic, closely linked to clinical medicine, sports science, and nutrition, is crucial for enhancing human health and characterizing compositional variations in health and disease states[2].

Although many modern scientists have significantly advanced the field as it is known today, interest in body composition dates back thousands of years, with early concepts originating from the Greeks. Around 440 BC, Hippocrates, often referred to as “the father of medicine”, theorized that the human body is composed of four key "constituents": blood, phlegm, black bile, and yellow bile[2]. Similarly, ancient Chinese scholars proposed that the body is composed of five "elements": metal, wood, water, fire, and earth, with health dependent on the balance of these elements[3]. Although these early ideas were not grounded in experimentation, they marked the beginning of humanity's journey toward understanding body composition. Since then, humans have made significant progress in understanding the composition of their own bodies. Indeed, over the past century, several assessment methods have been introduced based on major scientific breakthroughs[4]. Interestingly, four key families of methods trace their origins to Nobel-Prize-winning discoveries: Roentgen’s X-rays (1895), Becquerel’s radioactivity (1896), Urey’s identification of deuterium (1931), and Rabi’s development of nuclear magnetic resonance (1938)[4].

The field has experienced remarkable growth, with over 60,000 studies published and an average annual output of 797 publications[a]. From 1946 to 1999, the average number of articles per year was 180, whereas since 2000 it has risen to around 2,009 annually[a] (Figure 1.1). Over the last decade (2015-2025), 29,472 studies were published, averaging 2,679 per year.

[a] These data come from a PubMed search conducted on February 6, 2026, using the search terms “human” and “body composition”.

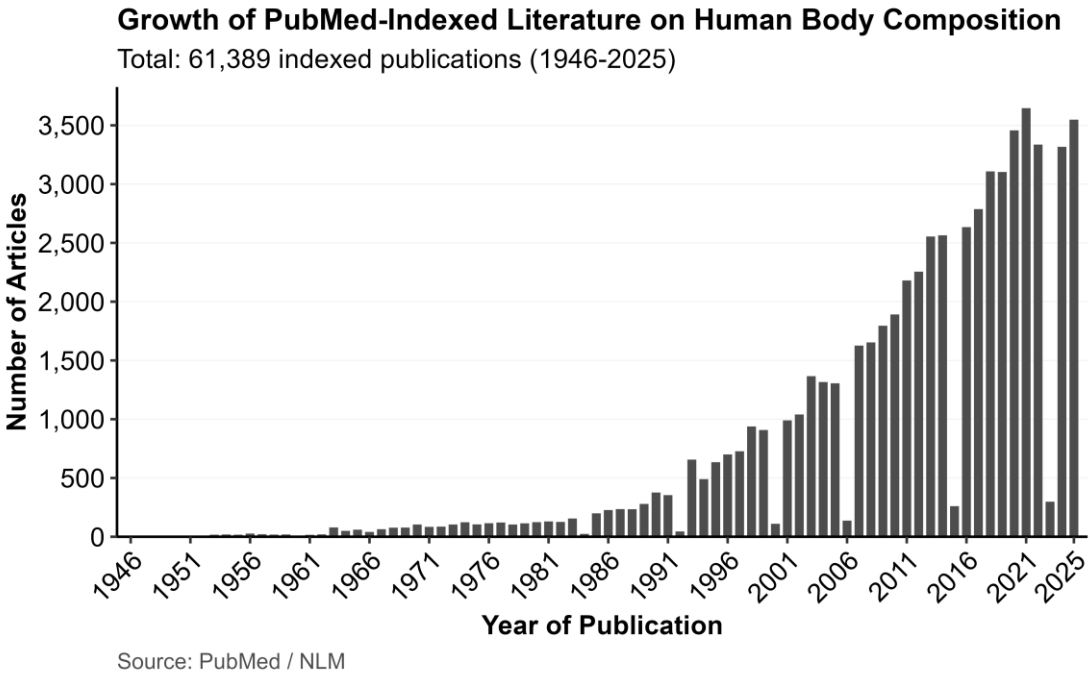

Figure 1.1 Temporal evolution of scientific publications indexed in the PubMed database for the search terms human AND "body composition" between 1946 and 2025.
Note: Due to low numerical frequency, records for the 1946-1952 period (fewer than 10 articles per year) are not visually discernible, although they are included in the total sample (N = 61,389).

The importance of body composition assessment

Adherence to the Nutrition Care Process (NCP) model is recommended in both clinical and field settings to ensure high-quality nutritional care. This systematic model consists of four distinct but interconnected steps[5]: 1) Nutritional assessment (including biochemical, clinical, dietary and body composition data); 2) Nutritional diagnosis; 3) Nutritional intervention; and 4) Monitoring/Evaluation. Within this framework, body composition assessment is fundamental. This assessment encompasses adherence to appropriate national or international technical-scientific norms, exemplified by the use of skinfold measurements according to the standards established by the International Society for the Advancement of Kinanthropometry[6] (ISAK). Moreover, it requires the ability to compare the obtained values with reference or normative data, a process critical in formulating appropriate decisions about the nutritional diagnosis (2nd step) and subsequent intervention (3rd step).

Specifically in sports nutrition, body composition assessment is crucial for several reasons[7]. First, it allows practitioners to track changes in an athlete's body composition, providing valuable feedback on the effectiveness of training programs and nutritional interventions. Second, understanding the specific physical characteristics critical to performance enables the optimization of training and dietary strategies. Third, for athletes in weight-category sports, determining safe and achievable body composition targets is essential for performance and safety. Additionally, monitoring body composition helps identify health issues associated with excessively low body fat levels, thereby reducing the risk of associated disorders. Finally, assessing body composition is vital for tracking growth, particularly in young athletes on talent development pathways, ensuring their development aligns with long-term performance goals.

Although assessing an athlete's body composition is crucial, practitioners often select a measurement method based on convenience and logistics rather than a comprehensive understanding of its methodological limitations[8]. Understanding the principles and limitations of body composition assessment methods is key to the cautious and critical interpretation of measurements. This knowledge also facilitates a comprehensive evaluation of body components, revealing changes in response to interventions like training and nutrition.

While many individuals view body composition primarily as the assessment of adiposity, it encompasses a comprehensive analysis of all components of the human body[9]. Consequently, it is essential to clarify key terminology and recognize that

existing assessment methods rely on different approaches to quantifying the body's constituent parts. For this reason, the following section introduces the main concepts of body composition, along with relevant models and measurement methods.

Assessment of body composition: levels, models, and methods

In 1992, to structure and systematize the study of human body composition, Wang and colleagues[3] introduced a comprehensive model of human body composition, delineating five distinct levels of ascending complexity. Each level encompasses clearly defined components that collectively contribute to the overall body weight. The five levels are: atomic, molecular, cellular, tissue-system, and whole-body (Figure 1.2).

The most frequently employed techniques for evaluating body composition across some of the five hierarchical levels will be discussed below.

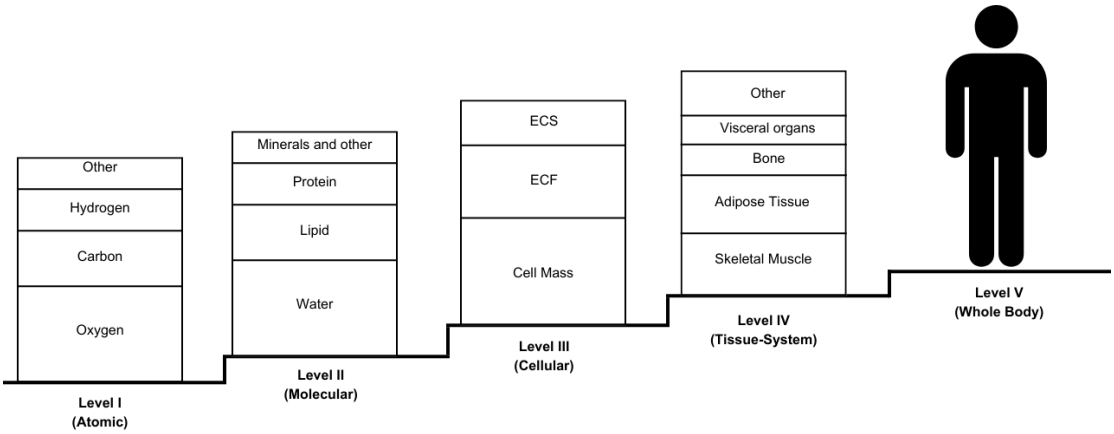

Figure 1.2 Five-levels of human body composition. ECS, extracellular solids; ECF, extracellular fluids[3].

Atomic-level

Atoms or elements form the fundamental building blocks of the human body. Approximately 96% of the body's mass comprises four key elements: oxygen, carbon, hydrogen, and nitrogen, which, along with an additional seven elements (sodium, potassium, phosphorus, chlorine, calcium, magnesium, and sulfur), comprise over 99.5% of the body mass[10].

Elemental analysis in humans has traditionally been conducted using cadavers or biopsy samples from specific tissues and organs. These elements can be assessed *in vivo* through neutron activation analysis[11] or whole-body counting[12] techniques. The assessment of these elements is relevant to the fields of radiobiology and nuclear medicine research.

Molecular-level

Most methodologies currently used in sports are applied at both the molecular and whole-body levels[13]. Elements are incorporated into molecules at the molecular

level, which consists of five major components: water, lipids, proteins, minerals and glycogen[3]. It is also possible to specify subfractions of components, such as lipids' fat or triacylglycerol portion, the extracellular and intracellular sections of total body water, and the minerals found in soft tissues and bone[14]. Figure 1.3. presents the different components at the molecular level.

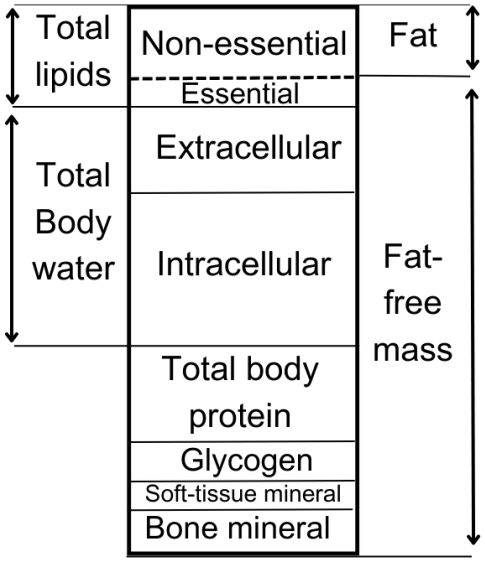

Figure 1.3 Molecular level components[10].

Water is the most abundant chemical substance in the human body, accounting for approximately 60% of body weight in the Reference Man[10]. Total body water (TBW) can be divided into intracellular (ICW, ≃ 65% of TBW) and extracellular water (ECW, ≃ 35% of TBW)[15].

Lipids are the most misunderstood of the five main molecular chemical components, as fat and lipids are sometimes used interchangeably, even though they conceptually do not correspond to the same components[16]. All biological material extracted using lipid solvents, such as ether and chloroform, is categorized as lipids[17]. Triacylglycerols, commonly referred as fat, are the most prevalent lipids in humans. However, some inaccurately use "fat" to describe all lipids, leading to confusion in the literature[14]. Non-fat lipids, such as phospholipids, sphingolipids, and steroids, are essential in biochemical and physiological processes, while triacylglycerols (fat or nonessential lipids) primarily serve as energy storage[14]. The widely accepted density of fat is 0.9007 g/cm³ at 36 °C[3, 18].

Protein in body composition research encompasses nearly all nitrogen-containing compounds, from amino acids to nucleoproteins[3]. In their dry crystalline state, most proteins have densities around 1.27 g/cm³[19]. However, proteins are key water-binding agents in the body, leading to solute and solvent volume

contraction[20]. The best estimate for the density of hydrated proteins in living cells is 1.34 g/cm³, although individual proteins may vary in specific density[18].

Minerals are distributed across two main compartments: bone minerals and non-bone or soft tissue minerals. The main component of bone minerals is calcium hydroxyapatite, with smaller contributions from sodium, potassium, magnesium, and chloride[21]. Soft tissue minerals include sodium chloride, potassium chloride, chloride ions, hydrogen phosphate, and bicarbonate[21]. The bone mineral density is 2.982 g/cm³. For soft-tissue minerals, densities vary from 3.07 g/cm³ for potassium bicarbonate to 4.99 g/cm³ for magnesium chloride. The mean overall density of soft-tissue minerals is 3.317 g/cm³, considering the relative contributions of each mineral[18].

Glycogen, the main storage form of carbohydrates, is located in the cytoplasm of most cells. However, it is predominantly stored in the liver and skeletal muscle, accounting for approximately 2.2% and 1.0% of their wet weights, respectively. The average density of glycogen is 1.52 g/cm³[3].

Molecular-level models

The most commonly applied molecular model in sports is the two-compartment model (2C), which includes fat mass (FM) and fat-free mass (FFM)[13]. Typically, 2C models assess body density (BD, also known as densitometric models) or TBW (referred to as hydrometric models). This model assumes a uniform FFM composition, disregarding the variations in TBW, protein mass, and bone mineral content (BMC). Therefore, it relies on certain assumptions, such as that the densities of FM and FFM being 0.9007 g/cm³ and 1.100 g/cm³[18], respectively, and the FFM/TBW ratio being ≃0.73[22, 23]. However, the FFM density assumption was based on analyses conducted on just three male cadavers[18] and the FFM/TBW ratio on nine adult human cadavers[23]. Consequently, deviations from these assumptions are possible due to factors such as aging, athletic status, and intentional weight loss [20, 24-26].

Densitometric models

Densitometric models estimate body composition from BD[27], which can be accurately measured in most children and healthy adults. Models based on molecular components rely on calculated and assumed constant densities of these components[14]. Any material's density is equivalent to its mass-to-volume ratio. Because body mass measurement is relatively straightforward, the prerequisite for precisely determining BD is obtaining an accurate measurement of body volume

(BV)[27]. BD is then used to calculate body fat percentage (%BF) using various equations, most commonly those proposed by Siri (1956)[28] and Brozek et al. (1963)[18].

BV[b] can be assessed in individuals using two techniques: hydrodensitometry (underwater weighing [UWW]) and air-displacement plethysmography (ADP)[27]. UWW is the most traditional method, requiring full-body submersion in water with subjects exhaling completely. The calculation of BD involves measuring the water displaced or, more commonly, the body's weight underwater[27]. ADP has emerged as a superior alternative to UWW due to its greater reliability and reduced invasiveness[29]. Notwithstanding, ADP volume assessments can be influenced by body temperature, moisture, facial hair, and clothing worn during testing[30, 31]. Also, both methods require determining or predicting residual lung and thoracic gas volumes[32].

Densitometric methods enable the estimation of FM using 2C models; however, in multi-component models, assessments of BV are also required[3]. The validity of assumptions underlying the conversion of BD to composition limits both UWW and ADP. When uncertainty arises regarding the fulfillment of these assumptions, it is advisable to use UWW and ADP in conjunction with other methods within the 4C model[27].

ADP is currently the most commonly used criterion method for determining BV. The ADP method estimates BV by measuring the volume of air displaced by an individual within a closed chamber, known as a plethysmograph[33, 34]. This system is composed of two complementary chambers (a reference chamber and a test chamber where the individual sits), separated by a moving diaphragm[27]. When a person sits inside the test chamber, they displace a volume of air equal to their BV. This BV is determined by initially measuring the volume of air in the empty chamber and then subtracting the volume of air remaining in the chamber with the subject inside[34]. This air is measured using Poisson's law, which relates pressure and volume under adiabatic conditions (*i.e.,* no heat transfer with the environment) [27].

Although BV measurement in the plethysmograph occurs mostly under adiabatic conditions, it is necessary to consider air maintained at isothermal conditions, such as air in the lungs, near the skin or hair, and in clothing[35]. Air under isothermal conditions is easier to compress. For a given volume and change in volume, the change in pressure will be lower under isothermal conditions[27]. The

[b] The author is aware that BV is formally a concept at the whole-body level (Level V). However, given that BV is employed in molecular-level multicomponent models, it was deemed more appropriate to address its measurement within this subsection.

effects from clothing and hair are minimized by wearing minimal clothing (a swimsuit) and a swim cap[34]. The measurements of raw BV are corrected for the average amount of air in the lungs during normal tidal breathing, thoracic gas volume (Vtg), and a surface area artifact[27]. Vtg can be directly measured or predicted using the Crapo et al. (1982)[36] models, especially when measurement is not possible or to save time. The surface area artifact correction is an estimate automatically calculated by the software using an empirical constant (k) and the subject's body surface area[34] (calculated from body weight and height with use of the formula by DuBois and DuBois[37]). In summary, a BV measurement by ADP consists of the measurement of the raw BV, calculation of the surface area artifact, and measurement/prediction of the Vtg[27, 35]:

BV = $raw\ BV + 0.40\ Vtg - SAA$

Where BV is body volume in litres, Vtg is the thoracic gas volume in litres and SAA is the surface area artefact in litres.

Hydrometric models

The human body predominantly consists of water[10]. Body water volume can be measured by isotope dilution using tracers such as tritium, deuterium, or ^{18}O-labeled water. With meticulous methodological considerations, TBW can be measured with precision ranging from 1% to 2%[38].

The principle behind hydrometric models is that fat is hydrophobic and thus free of water, which is therefore restricted to the FFM compartment[38]. The calculation of FFM from TBW depends on the assumption of constant hydration of FFM[38]. The most frequently employed hydration constant is 0.73, initially recommended by Pace and Rathbun[39] in 1945. This proposed hydration constant has been examined in both *in vitro* and *in vivo* studies. Regarding *in vitro* data, chemical analyses of nine adult human cadavers yielded an average TBW/FFM ratio of 0.737 (SD = 0.036), with a range of 0.684 to 0.808[23].

Isotope dilution

The criterion method for determining TBW is by dilution with tritiated water (^3H_2O), deuterium water (^2H_2O), or ^{18}O-labelled water ($H_2{}^{18}O$), followed by measuring the isotope enrichment in saliva, urine, or serum samples[27, 40]. In validation studies, these techniques are used as a criterion against alternative techniques.

In brief, the process of measuring TBW can be described in the following steps[41, 42]:

1. Baseline biological sample: A sample is collected to determine the baseline concentration of the isotope in the body.
2. Dose administration: The individual ingests a known and weighed amount. A deuterium-labelled water dose is most commonly used.
3. Equilibration (enrichment): It takes three to four hours to equilibrate across the body water pool[27].
4. Second biological sample: Following this time period, a biological sample is collected (with most studies using a period between 3 and 6 hours[43]).
5. TBW is estimated by applying the dilution principle[42]:

$$F1N1 = F2N2$$

$$N2 = F1N1/F2$$

The relationship between the tracer amount (N1), its enrichment (F1), and the enrichment after equilibration (F2) allows calculation of the total volume of the distribution space (N2).

Although these tracers are widely distributed throughout the body, they are not flawless tracers. Adjustments are needed for exchange with non-aqueous oxygen or hydrogen[27]. Indeed, there are four assumptions: (i) the tracer is distributed only in body water, (ii) it is equally distributed within this pool, (iii) it has a rapid equilibration period, and (iv) it remains unmetabolized throughout the equilibration period[41].

Multicompartment models

Recognized variations in hydration and bone mineral density among individuals led to the development of multicompartment models. Although we can find up to six molecular-level components, the most commonly used are the three-compartment (3C) and four-compartment (4C) models. These models were designed to accommodate interindividual differences in FFM components. Due to their incorporation of a greater number and variety of measured properties or components compared to the standard 2C models, these methods typically provide a more comprehensive representation of biological variability[22].

Multicompartment models are those in which the human body is divided into three or more molecular-level components[20]. Given that these models encompass a greater number and variety of measured properties or components than 2C models, they capture a broader range of biological variability[20]. For example, incorporating TBW into measurements minimizes errors in a traditional 2C model by accounting for potential fluctuations in FFM hydration[40]. The improvement continues by subdividing FFM into bone mineral mass and protein/residual, resulting in a 4C model. As a

result, multicompartment models are frequently chosen as the reference against which other methods are evaluated[20].

Water is the largest component with the lowest density among the FFM constituents, so it significantly influences FFM density[22]. Siri addressed the limitations of the 2C model with crucial contributions in 1956[28] and subsequent refinements in 1961[44], integrating total body water into the existing 2C molecular-level model, resulting in a comprehensive 3C model that considers fat, water, and non-fat solids (mineral and protein, nowadays termed residual mass)[45]. While Siri's is the most frequently used three-compartment model, other models have emerged[22].

Siri faced a limitation in his 3C model when he could not distinguish the residual mass component into its protein and mineral constituents due to the lack of suitable measurement approaches at that time[45]. However, dual photon absorptiometry and its successor, Dual-energy X-ray absorptiometry (DXA), marked a pivotal advancement in body composition research. These technologies enabled the *in vivo* measurement of bone mineral mass[46]. Consequently, Siri's model evolved into a 4C model by separating the main residual mass components into fat, water, bone minerals, and residual components[45].

Dual-energy X-ray absorptiometry (DXA)

DXA is the method of reference for measuring bone mineral density (BMD) at the lumbar spine and proximal femur[47]. Although initially developed to measure BMD, DXA technology has now been applied to assess whole-body composition. DXA is becoming one of the most widely used methods at the molecular level due to its low radiation exposure, cost-effectiveness, and rapid results[48]. As a 3C molecular model, DXA enables quantification of FM, lean soft tissue (LST), and BMC[48]. It also provides values for both regional and total body composition.

Historically, DXA technology has been primarily produced by three commercial manufacturers: Lunar (GE Healthcare), Hologic, and Norland (Swissray), although additional manufacturers have since entered the market. Each system utilizes an X-ray source emitting two energies (typically 40 kV and 70 kV), a detector, and interfaces with a computer system to image scanned areas[48]. Differential attenuation of the X-ray energies by the subject is employed to determine body composition[48]. The X-ray source is positioned below the scanning bed and measurements typically take 5 to 20 minutes, depending on the scan type and chosen software. Importantly, the radiation dose to the subject is minimal[48], ranging

from 0.02 to 1.5 mrem for a whole-body scan, depending on the instrument and scan speed[49].

DXA relies on several assumptions. Firstly, FM is derived from the assumed constant attenuation of pure fat and LST[49]. Secondly, measurements are presumed to be unaffected by the anteroposterior thickness of the body[49]. A third critical assumption pertains to the region of the body analyzed to gather composition data and how much fat content in the analyzed area correlates with the fat content in the unanalyzed regions[49]. Notably, in a typical whole-body scan, an estimated 40% to 45% of the 21,000 pixels contain both bone and soft tissue[49]. Consequently, these pixels are excluded from the calculation of soft tissue values. This exclusion poses a challenge, as the composition in areas where bone is detected must be extrapolated from surrounding regions containing only soft tissue[48]. Several factors influence DXA results, including the subject's thickness and size, calibration procedures, software version, and the specific company and model of the instrument[49].

Multicompartment models – Four-compartment (4C) models

In 4C models, four parameters are measured: BV, TBW, bone mineral, and body mass[20]. The 4C model integrates measurements from DXA for BMC assessment, from deuterium dilution for TBW measurement, and from ADP or hydrostatic weighing for determining BV.

They adhere to consistent fat, water, and bone mineral densities. The formula for the 4-component model can be generated as[22]:

$$\frac{1}{BD} = \frac{FM}{FM\ D} + \frac{TBW}{TBW\ D} + \frac{Mo}{Mo\ D} + \frac{Res}{Res\ D}$$

BD, body density; FM, fat mass; TBW, total body water; Mo, bone mineral; Res, residual; D, density.

Body volume models are based on the assumption that the densities of the components involved are known and constant *in vivo*. Their development employs two primary strategies: one assumes that the remaining body mass, post-subtraction of fat, water, and bone minerals, comprises known-density protein and soft tissue minerals, while glycogen is ignored. Alternatively, the other approach speculates that the remaining mass is a known-density combined residual mass encompassing protein, soft tissue minerals, and glycogen[20].

Table 1.1 outlines several frequently employed 4C models. These methods generally provide comparable total body fat estimates due to their reliance on closely related models and assumptions[20].

The primary challenge lies in modelling small, unmeasured components, such as soft tissue minerals, glycogen, and other residual mass components[45]. Various models exist for deriving soft-tissue mineral mass, each with its unique strategy. For instance, Selinger (1977)[50] assumed a constant fraction (1.05%) of body weight for soft tissue minerals, while Heymsfield et al.[51] assumed a constant fraction (0.235) of bone mineral "ash". Ash is generated when bone mineral is heated to temperatures exceeding 500 °C, releasing labile elements. It is essential to differentiate between 'bone ash' and bone mineral, as during the heating process, 1 g of bone mineral yields 0.9582 g of ash[45]. However, these models lack a robust mechanistic basis, as non-osseous minerals are distributed in both extracellular and intracellular water compartments within soft tissues, not forming 'constant' ratios to body weight or bone mineral mass[45].

Table 1.1 Example of 4C models for estimating total body fat mass.

Author	Equation	Notes
Selinger (1977)	FM (kg) = 2.747 BV - 0.714 TBW + 1.129 Mo - 2.037 BM	Ms = 0.0105 BM
Heymsfield et al. (1990)	FM (kg) = 2.747 BV - 0.714 TBW + 1.129 Mo - 2.037 BM	Ms = TBBA 0.235
Baumgartner (1991)	FM (kg) = 2.747 BV - 0.7175 TBW + 1.148 M - 2.05 BM	Ms = 0.235 Mo
Fuller et al. (1992)	FM (kg) = 2.747 BV - 0.710 TBW + 1.460 TBBA – 2.05 BM	Ms = TBBA 0.23048
Friedl et al. (1992)	FM (kg) = 2.559 BV – 0.734 TBW + 0.983 Mo – 1.841 BM	Residual mass (Prot + Ms + Gly) with a density of 1.39 g/cm^3
Withers et al. (1992)	FM (kg) = 2.513 BV – 0.739 TBW + 0.947 Mo – 1.790 BM	Residual mass (Prot + Ms + Gly) with a density of 1.404 g/cm^3
Wang et al. (2002)	FM (kg) = 2.748 BV – 0.699 TBW + 1.129 Mo – 2.051 BM	Ms = 0.0129 TBW

Abbreviations: FM, fat-mass; BV, body volume; BM, body mass; TBW, total body water; M, total mineral; Mo, bone mineral; Ms, soft mineral; TBBA, Total-body Bone Ash; Prot, protein; Gly, glycogen.

In 2002, Wang et al.[52] highlighted the limitations of existing 4C models, noting the lack of an accurate estimate of soft-tissue minerals. Despite its relatively small mass in adults (about 400 g), the contribution of soft tissue minerals to body density is significant, given their higher density (3.317 g/cm^3) compared to other components like fat (0.9007 g/cm^3), water (0.99371 g/cm^3), protein (1.34 g/cm^3), and bone mineral (2.982 g/cm^3). Wang et al.[52] presented equations for predicting soft tissue mass and introduced a five-component model relying on ECW and ICW, derived through a combination of labelled water and bromide dilution volumes, later simplified to a 4C model (see Table 1.1), assuming soft Ms can be estimated from TBW (Ms = 0.0129 TBW)[45].

While measuring more components enhances validity, there is concern that greater control over the biological variability of FFM might be compromised by the propagation of errors in determining body volume, TBW, and Mo[20]. A worst-case scenario for error propagation can be calculated by assuming that the squared standard errors of measurement (SEM²) or squared technical errors of measurement (TEM²) are independent and additive[20]. These assumed additive errors do not appear to compromise the improved accuracy of fat estimations[22, 52].

DXA-derived BV

In the last decade, several studies[53-55] have introduced a novel method for quantifying BV using DXA. Eliminating the requirement for UWW or ADP simplifies the development of multicompartment models, thereby improving accessibility in both research and field settings[56]. Heymsfield et al. (2015) considered the introduction of DXA-derived BV as one of the milestones in the development and implementation of multi-component body composition models[45]. Currently, no DXA-derived BV equation has been developed for athletes. Additionally, Heymsfield et al. (2021) discussed an alternative approach to BV estimation that aligns with the longstanding theoretical foundation of multicomponent body density models[56]. This theory-based approach can be described as follows:

$$\text{DXA-BV (L)} = \left(\frac{FM}{0.9007}\right) + \left(\frac{LST}{D\ LST}\right) + \left(\frac{Mo}{2.982}\right)$$

where 0.9007 kg/L represents the density of FM and 2.982 kg/L represents the density of Mo[45]. LST, unlike FM and Mo, is a heterogeneous component that includes TBW, Ms, protein, and small amounts of Gly. Heymsfield and colleagues[56] have suggested a provisional value of 1.064 kg/L for the LST density, however athletes were not included in any of the samples used in its derivation.

Cellular-level

While the human body can be broken down into various molecular components, its organization into cells is what forms a living organism. At the cellular level, the body consists of three primary compartments: cells, extracellular fluid, and extracellular solids[3]. Limited research has been conducted at this level, likely due to challenges in accurately quantifying certain components[3].

Tissue-system level

The tissue-system level components include adipose tissue, skeletal muscle, bones, visceral organs, and the brain[14]. In the 19th century, anatomists quantified

body composition at the tissue-system level through cadaver analysis[57]. By the early 20th century, numerous studies on organ and tissue weights had provided a reasonably accurate understanding of the weights and distributions of tissues and organs that constitute the main components of body mass[3, 58, 59]. The advent of imaging techniques, such as computed tomography (CT) and magnetic resonance imaging (MRI), has revolutionized the study of body composition, transitioning it from 19th-century methods to modern practice[57]. These imaging methods are now considered the most accurate for *in vivo* quantification at the tissue-organ level, despite challenges related to cost and access[60]. Widely used in body composition research, CT and MRI are essential for calibrating field methods and are uniquely capable of measuring the composition of internal tissues and organs, including skeletal muscle and liver tissue[60].

Other indirect methods for estimating body composition at the tissue-system level include DXA and anthropometric techniques. Currently, models are available to estimate skeletal muscle mass from appendicular lean soft tissue (ALST) measured by DXA[61, 62]. Another practical alternative involves using equations based on anthropometric variables, such as circumferences and skinfolds, to estimate skeletal muscle mass[63].

Whole-body level

The whole-body level, the fifth tier of body composition analysis, focuses on overall body size, shape, and external physical characteristics[3]. This level involves several dimensions, with commonly assessed metrics including height, body mass, segment lengths, circumferences, skinfold thickness, BV and body density[3].

The interrelation between variations observed at the whole-body level and changes in body composition at the other four levels provides a basis for estimating their components using whole-body measurements[3]. Since whole-body indicators are generally more straightforward and accessible to conduct than those at other levels, these techniques are particularly suitable for real-life settings, such as professional sports[3, 32].

Anthropometry

Anthropometry encompasses a range of measurements, including body mass, standing height, breadths, circumferences, and skinfold thickness, all of which require standardized techniques to ensure accuracy[6, 64].

The most common method in the sports industry for body composition assessment has been to collect data on skinfolds and girths[13] due to the technique's

simplicity, low cost, speed, precision and its ability to be used more frequently[8]. Compared with other frequently used field-level methods, such as bioelectrical impedance, skinfold assessment is less affected by everyday activities, meal ingestion, and changes in hydration status[8, 65]. The skinfold method involves using a calliper to measure a double fold of skin at various sites, providing an estimate of subcutaneous fat[66]. While over a hundred equations have been developed to convert skinfold measurements into body density or FM, these estimates are based on several critical assumptions: 1) consistent compressibility of skin and subcutaneous fat, 2) uniform skin thickness, 3) a stable fat fraction within adipose tissue, 4) uniform adipose tissue distribution, and 5) a fixed ratio of visceral to subcutaneous fat[66].

Classical regression equations, such as those developed by Jackson and Pollock[67, 68], remain widely used for predicting FM[40]. These equations are based on body density measurements obtained by hydrodensitometry, with FM calculated using Siri's equation[28]. However, as previously discussed, the densitometric method has inherent limitations, particularly concerning the hydration and mineral content of FFM, which can lead to inaccuracies in FM estimation. Subsequently, skinfold regression equations derived from four-compartment models have been introduced (Peterson et al. 2003[69]; Evans et al. 2005[70]), offering potentially greater accuracy in body composition assessments[40]. Regarding the athletic population, a 2024 systematic review by Serafini et al.[71] showed that most anthropometric equations were developed using DXA as the reference, with only one study using a 4C model[70].

An alternative approach is to use the sum of skinfolds as a direct indicator of adiposity[8, 32]. This method allows comparison with published values for athletes[8, 72] and is increasingly advocated and used for field evaluation of adiposity in sports nutrition[8, 13, 73]. A 2013 survey conducted by the Ad Hoc Research Working Group on Body Composition, Health, and Performance under the IOC Medical Commission revealed that over 50% of 216 sports professionals from 33 countries reported using the sum of skinfolds following the ISAK protocol for body composition assessment[13]. The advantage of using skinfold thickness as a valid measure, without converting it to fat mass or body fat percentage, is that it avoids the assumptions inherent in such conversions. A 2022 update on the previously mentioned survey indicated that surface anthropometry remains the most commonly used method and that 40% fewer professionals are using equations to convert skinfolds to FM%, preferring the sum of skinfolds[73].

Bioelectrical impedance

Bioelectrical impedance is commonly used to estimate body composition using a 2C body composition model. Available research examining body composition practices in sports shows that approximately one-third of surveyed practitioners and scientists reported using BIA in both 2013 and 2022[13, 73].

In brief, bioelectrical impedance analysis (BIA), regardless of the device used, involves applying a weak, alternating electrical current at one or more frequencies through leads connected to surface electrodes[74, 75]. This process characterizes the body's conductive and nonconductive tissues and fluids.

Electrodes can be attached to the body in various configurations. In the conventional whole-body BIA approach, adhesive electrodes are placed on the dorsal surfaces of the right foot and ankle and the right wrist and hand, while the subject is in a supine position; however, standing devices utilizing metal electrodes on both hands and feet are also common[40, 75]. Raw BIA data between positions should not be considered interchangeable[76, 77].

BIA relies on the principle that the body's water content, which conducts electrical currents, is closely related to the body's composition. Tissues offer two types of resistance: resistance (R) and reactance (Xc)[74]. R is the opposition to the passage of an alternating current through intra- and extracellular fluids, being inversely associated with their content[74]. At the same time, Xc represents the additional resistance caused by cell membranes and tissue interfaces[78]. Geometrically, impedance (Z) is the vector composed of R and Xc and is expressed in Ohms (Ω)[78]. Capacitance is a function of Xc that arises when cell membranes store part of the current, creating a phase shift or phase angle (PhA) – which is derived as the angular transformation of the reactance-to-resistance ratio (arctangent [Xc/R] × 180 / π) and expressed in degrees[75, 78].

Several BIA devices are available, including single-frequency (SF-BIA), multi-frequency (MF-BIA), and bioelectrical impedance spectroscopy (BIS) devices. SF-BIA devices, operating at 50 kHz and utilizing a wrist-ankle tetrapolar electrode configuration, are the predominant bioimpedance methodology[75]. At this frequency, the current passes through both intra- and extracellular fluids, although the proportions may vary[74, 79]. However, SF-BIA primarily reflects the ECW space[74, 79].

The measured impedance can be converted to a commonly evaluated body composition component – TBW –, by the following equation or derivations by empirical equations adjusted for variables such as height and weight[78, 80]:

$$V = \rho \frac{L^2}{R}$$

ρ is the specific resistivity of body fluids (ohm x cm), L is the length of the conductor, e.g., height (cm), and R is the electrical resistance of the conductor (ohm).

This volumetric equation presupposes that the conductor possesses a uniform cylindrical shape and that the current is uniformly distributed across the conductor[81]. The unequal impact of body segments on impedance measurements is another limitation[82]. For instance, the torso accounts for 5 to 12% of the body's total impedance and almost half of the overall weight[83]. Therefore, impedance will be less affected by changes in torso hydration than by comparable changes in limb hydration[74].

From the raw BIA data (R, Xc, PhA and Z), and considering many assumptions (*e.g.,* typically assuming a 0.73 hydration fraction for FFM)[74, 75, 78, 84, 85], it is possible to estimate several body components, depending on the selection of an appropriate predictive equation. The impedance index (L^2/R) is commonly used in regression equations with additional variables in which BIA and anthropometric data are regressed against reference-determined TBW or FFM[40, 74]. However, many devices do not specify the programmed equation. Therefore, when raw data are not provided, body composition estimation, including fluid content, depends on the manufacturer's algorithms[75].

MF-BIA requires the use of at least two frequencies. Depending on the current frequency, the electric current flows differently through the ECW and the ICW. In MF-BIA, a low current (commonly around 5 kHz) is combined with multiple higher frequencies (such as 50, 100, 200, or 500 kHz)[74, 75]. This approach enables the estimation of ECW and ICW, as low frequencies primarily reflect ECW, while higher frequencies approximate TBW, with ICW calculated as the difference[74, 78]. Like SF-BIA, MF-BIA uses empirical linear regression models to predict body compartments.

In contrast, BIS uses a range of frequencies to calculate impedance, allowing extrapolation of resistance at zero (Re) and infinite ($R\infty$) frequencies, which correspond to ECW and TBW, respectively. It is not feasible to accurately predict ECW impedance readings at a very low, ideally zero, frequency[79]. Therefore, the BIS technique employs a method called Cole Modelling to make measurements across a range of frequencies and to extrapolate these results to zero and infinite frequencies[79]. The Cole model variables are subsequently utilized in equations constructed from the Hanai mixture theory[86], which describes how electrical characteristics of tissues are altered by the combined impact of conducting components (water, electrolytes, lean tissue) and nonconducting components (bone,

fat) within the body[75, 87]. Unlike MF-BIA, which estimates ICW by subtracting ECW from TBW, BIS directly measures ICW and ECW and sums them to obtain TBW. Additionally, BIS does not depend on population-specific prediction equations derived from regression statistics[75].

Organization of body composition methods

While the previous section described several methods at each body composition level, the literature lacked a systematic organization, with some authors grouping methods into categories such as “direct”, “indirect”, and “doubly indirect”. In order to move beyond simple methodological lists and this “direct-indirect” categorization, Wang and colleagues[88] developed a classification system, which begins with the division into *in vitro* and *in vivo* categories, advancing to the nature of the measurable quantity (property, component or combined) and ending with the mathematical function employed (Type I or Type II).

Figure 1.4. shows the proposed organization of body composition methods, as proposed by Wang et al.[88].

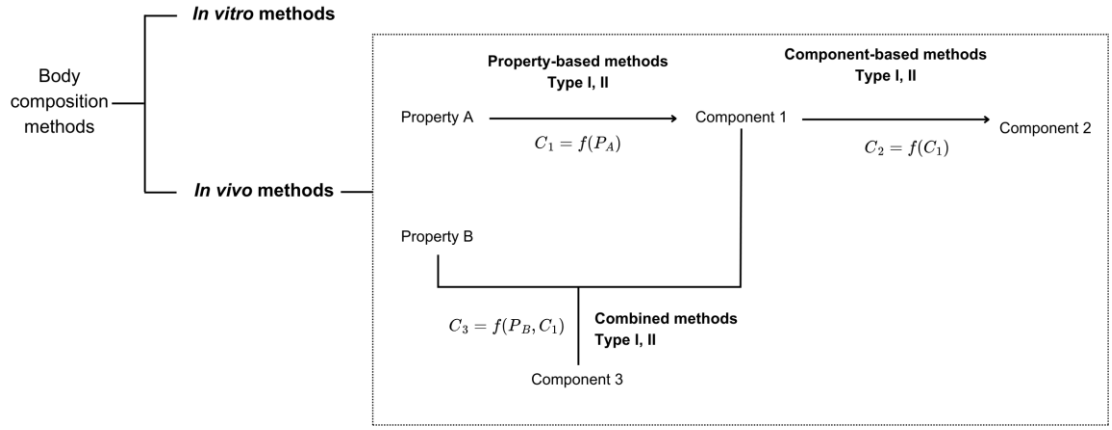

Figure 1.4 Suggested classification of body composition methods by Wang et al.

Body composition methodology can be broadly categorized into two areas*: in vitro* and *in vivo* methods[88]. *In vitro* methods involve measuring components in whole cadavers or excised tissues.

All *in vivo* human body composition methods can be summarized by a basic formula[88]

$$C = f(Q)$$

In this formula, C denotes an unknown component, Q represents a measurable quantity, and f is the mathematical function that links Q to C. The formula illustrates that quantifying an unknown component relies on two interrelated aspects: the measurable quantity and the mathematical function.

Body composition methods rely on two primary types of quantities: properties and components. These form the basis of property-based and component-based methods, respectively, while "combined" methods incorporate both types as

measurable quantities. Additionally, two types of mathematical functions (type I and type II) are used, resulting in six categories of methods that encompass all available in vivo techniques[88].

Organization by measurable quantity

All *in vivo* methods can be classified into one of three categories based on the measurable quantity: property-based, component-based, and combined methods.

Property-based methods: quantify an unknown component by measuring a specific property, which must distinguish the component from others[88]. Examples of measurable properties include X-ray attenuation and skinfold thickness; in some cases, multiple properties are used together, as in hydrodensitometry, where body volume and weight are combined to quantify FM and FFM[18].

Component-based methods: quantify an unknown component by using known components derived from property-based methods[88]. A key aspect of these methods is that a property-based method must first estimate the known component. The principle behind component-based methods is that a stable or relatively stable quantitative relationship exists between the unknown and known components[88]. For example, FFM can be estimated from TBW by assuming that 73% of FFM in healthy adults is water[38].

Combined methods: the quantities used to estimate unknown components include measurable properties and known components. An example of a combined method is Siri's method, which quantifies total body fat using two measurable properties (body weight and volume) along with a known component, total body water[89].

Property-based methods form the basis of *in vivo* techniques, with component-based and combined methods expanding upon this foundation. Consequently, all *in vivo* methods rely on the measurement of quantifiable properties[88].

Organization by mathematical function

Type I methods involve a mathematical function derived from the statistical analysis of quantitative measurements, typically requiring a reference method to quantify the unknown component[88]. Statistical techniques, often regression analysis,

are used to create an equation that predicts the unknown component based on a property or a property-derived component[88]. For example, total body fat can be predicted from skinfolds and circumferences, measured with a skinfold calliper and anthropometric tape, using the four-compartment model as the reference method (e.g., Evans' equation)[70]. Since type I methods are population- and condition-specific, they must be revalidated when applied to new populations or different conditions[88]. For example, a DXA-derived BV prediction equation developed in overweight adults may not apply to athletes.

Type II methods: characterized by mathematical functions that use constant or relatively constant ratios or proportions[88]. These methods often depend on specific assumptions made when developing the models, and their validity largely hinges on these assumptions. An example is the measurement of FM based on a molecular-level 2C densitometric model. This Type II method assumes the FM and FFM densities are constant at 0.9007 g/cm³ and 1.100 g/cm³, respectively[18]. The current reference for FM assessment, the 4C model, is also a Type II method. Developing this model required some assumptions, though they are relatively minor.

Type I methods can be developed for any measurable quantity if a strong correlation exists between the unknown component and the quantity[88]. In contrast, Type II methods require models based on constant or relatively constant ratios or proportions, thereby restricting their development. Consequently, there are fewer Type II methods compared to the numerous Type I methods currently in use[88].

Molecular-level body composition assessment in athletes

Two-compartment models

The most commonly used molecular model in sports is the 2C, which includes fat mass (FM) and fat-free mass (FFM)[13]. However, as previously noted, 2C models rely on certain assumptions, such as that the densities of FM and FFM being 0.9007 g/cm³ and 1.100 g/cm³[18], respectively, and the FFM/TBW ratio being ≃0.73[23]. However, it has been demonstrated that athletes deviate from these assumed constants[24, 25]. Regarding *in vivo* data, current evidence indicates that FFM hydration and density in athletes, as measured by dilution methods and multicompartiment models, often deviate from the assumed constant[25, 90-94]. Table 1.2 summarizes key studies evaluating FFM hydration and density in athletes using dilution methods and multicompartment models.

Table 1.2 FFM hydration and density in studies with athletes using dilution methods and multicompartment models.

Reference	**Sample size/sex/sport**	**Age, years** Mean (SD)	**TBW/FFM, %** Mean (SD)	**FFM_D, g/cm³** Mean (SD)
Penn et al. (1994)[95]	10 male recreational long-distance runners	27.9 (6.8)	73.5 (0.8)	1.098 (0.003)
Lichtenbelt et al. (2004)[94]	16 male bodybuilders	32.7 (NA)	72.6 (1.2)	1.099 (0.004)
Silva et al. (2006)[93]	46 male adolescent athletes	15.3 (1.2)	72.4 (1.7)	1.100 (0.007)
	32 female adolescent athletes	15.1 (0.3)	70.9 (1.2)	1.105 (0.004)
Santos et al. (2010)[92]	27 elite male judo athletes, period of weight stability	22.2 (2.8)	72 (2)	1.100 (0.007)
	27 elite male judo athletes, prior to competition	22.2 (2.8)	71 (3)	1.102 (0.009)
Santos et al. (2014)[25]	12 male basketball players pre-season	16.2 (0.6)	71.3 (2.8)	1.101 (0.010)
	11 female basketball players, pre-season	16.3 (0.5)	72.4 (1.6)	1.099 (0.005)
	12 male basketball players, competitive period	16.9 (0.8)	70.6 (0.5)	1.103 (0.002)
	11 female basketball players, competitive period	16.7 (0.6)	69.9 (1.7)	1.108 (69.9)
Sagayama et al.	58 collegiate athletes	20.3 (1.5)	72.1 (1.5)	NA

(2020)[91]				
Matias et al. (2021)[96]	72 male athletes (development sample)	23.2 (5.0)	74.1 (NA)	NA
	23 female athletes (development sample)	22.1 (4.6)	72.1 (NA)	NA
	33 male athletes (validation sample)	21.9 (4.7)	74.4 (NA)	NA
	14 female athletes (validation sample)	24.9 (6.0)	72.7 (NA)	NA
Cataldi et al. (2023)[90]	35 male collegiate athletes	24.4 (5.1)	70.0 (2.1)	NA
	35 female collegiate athletes	21.9 (4.2)	69.8 (2.9)	NA

Abbreviations: SD, standard deviation; TBW, total body water; FFM, fat-free mass; NA, not-available; FFM_D, fat-free mass density.

DXA (three-compartment model)

While DXA is not universally available in all field settings, a recent follow-up to a 2013 international survey on body composition practices in sports reported an 11% increase in DXA use from 2013 to 2022, highlighting its growing importance in the field[73].

Besides being one of the methods required in a criterion 4C model, DXA has the advantage of providing estimates of regional body composition. Regional analysis can help identify asymmetries and imbalances that may affect performance or increase injury risk, especially in sports that involve more extensively used body parts or sides[97, 98]. Monitoring regional changes also facilitates the identification of FFM loss or FM gain post-injury, informing focused rehabilitation and clinical decisions[98]. Additionally, DXA's capacity to measure BMC is a key strength for athletes' assessment. This measurement is becoming increasingly relevant as awareness grows of the consequences of low energy availability (mismatch between energy intake and the energy demands of exercise, compromising health and performance[73]) for bone health[99]. A 2024 systematic review and meta-analysis reported the prevalence of low energy availability in athletes to be approximately 45%[100]. Among the several negative consequences, impaired bone health is a primary concern associated with prolonged/severe exposure to low energy availability[101].

Performing a whole-body DXA scan involves three crucial phases: subject preparation, careful positioning, and post-processing of the scan data[102]. Although most focus is on subject presentation[103] and consistent patient positioning[104], careful post-scan analysis is also essential for maximizing precision[105]. During post-scan analysis, regional composition estimates (i.e., left and right arms, legs, and trunk) are determined by automatically or manually placing regions of interest (ROIs). Previous studies have primarily compared automatically and manually defined ROIs [106, 107]. However, the reproducibility of ROI measurements in DXA scans, particularly

regarding intra- and inter-measurer reliability, remains underinvestigated. Furthermore, existing investigations were conducted on older Lunar[108] or Hologic[109] scanners, with no examination to date of the latest generation of DXA equipment with more advanced software.

Four-compartment (4C) models

While these methods significantly increase the accuracy of body composition assessments, they are technically complex, time-intensive, costly, less accessible, and burdensome for participants[56], limiting their use. Literature applying the criterion multicompartment models in athletes remains sparse, with most relying on them as criterion measures to validate more accessible field techniques[92, 93, 110-112].

In recent decades, research into body composition has been driven by technological advances, with both the public and researchers seeking ever-greater accuracy and precision[113]. A 21st-century example is the widespread popularity of smartphones. This has also impacted the field of body composition, making it now possible to obtain both anthropometric variables and FM estimates using the smartphone camera[114-116]. Wearable devices – such as watches and wristbands – are also now widely used, with the American College of Sports Medicine identifying wearable technologies as the "#1 fitness trend" for 2026[117]. Wearable technology continues to evolve, with smartwatch-based BIA offering the possibility to estimate body composition[114, 118]. Moreover, Artificial Intelligence has become a prominent topic, and its potential extends to assessing body composition[116, 119]. These technological advances offer promising tools for field-based assessments, with the potential to increase accuracy and precision.

Undoubtedly, there will continue to be a demand for easier, less expensive, and more practical methods[113]. Consequently, establishing the accuracy of current and novel technologies is critical for reliably informing clinical decisions and enhancing athletic outcomes[120].

The introduction and validation of DXA-derived BV[53-55] eliminate the need for UWW or ADP. A simplified version of the 4C model can be performed[121, 122] using BIS for TBW estimation and DXA for BV determination[53, 54]. This methodological simplification makes multicompartment models vastly more accessible in both research and field settings[56]. However, previous studies have relied on DXA-derived BV equations developed in non-athletic populations[53-55], and a critical gap remains regarding the athlete-specific validation of rapid 4C models.

The aims of this investigation

This dissertation includes four research studies in the field of Body Composition.

A novel method for quantifying BV using DXA measurements has been introduced in the past decade. However, as a type I method, currently available equations are population-specific. Within this context, **study 1** (chapter IV) aimed to develop and validate a DXA-derived BV equation in athletes, utilizing ADP as the criterion. Additionally, the performance of the new equation was compared to existing empirical and theory-based approaches for DXA-derived BV.

Study 2 (chapter V) was conducted to assess the cross-sectional accuracy of a rapid 4C molecular-level model by replacing deuterium dilution TBW measurements with bioelectrical impedance, alongside DXA-derived estimates of BV in athletes. Also, study 2 aimed to externally validate the DXA-derived BV equation developed in study 1 ($DXA\text{-}BV_{Silva}$) in an independent sample of athletes using ADP as the criterion.

While rapid 4C models have demonstrated cross-sectional validity, to the best of our knowledge, their accuracy for tracking changes over time remains unknown. Therefore, **study 3** (chapter VI) aimed to evaluate the validity of utilizing DXA-derived estimates of BV and BIS-derived TBW in a rapid 4C body composition model to track FM changes in athletes.

An international follow-up survey on body composition practices in sports reported a 11% increase in DXA use from 2013 to 2022, highlighting its growing importance in the field. Besides becoming increasingly relevant as awareness grows regarding the consequences of low energy availability on bone health, the ability to assess regional body composition is another key strength of DXA. **Study 4** (chapter VII) aimed to assess the intra-tester and inter-tester reliability of DXA in delineating regions of interest (ROIs) for measuring regional FM, LST, and BMC. Additionally, the prevalence of inconsistencies between automatically defined ROIs and manufacturer guidelines was also investigated.

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Chapter III - Methodology

This chapter provides a brief description of the methodology used. A detailed description of each methodology is presented in the individual studies that constitute this dissertation.

Participants and study design

Studies 1, 2 and 3 (chapters IV to VI) were conducted using combined data from projects funded by the Portuguese Foundation for Science and Technology (FCT) ("Rapid Weight Loss, Body Composition, and Physical Performance Changes in Judo Athletes", grant PTDC/DES/69495/2006; "Body Composition and Physical Performance Changes Over a Season in Elite Athletes", grant PTDC/DES/098963/2008; and grant UIDB/00447/2020) and collected during PhD scholarships from FCT (2020.05397.BD and 2021.07122.BD) from the Exercise and Health Laboratory, CIPER, Faculty of Human Kinetics, University of Lisbon[1-5]. The first two projects used an observational design with a follow-up over the season and were used in Studies 2 and 3. The remaining projects used a cross-sectional design and were utilized in Study 1.

The author of the current dissertation collected data for **Study 4** (Chapter VII) from December 2023 to May 2025. For this observational study with two data collection moments, all procedures were carried out from study conception through data analysis, including the development of a detailed study protocol that was approved by the Ethics Committee of the Faculty of Nutrition and Food Sciences of the University of Porto (153/2023/CEFCNAUP).

In Table 2.1 the number of athletes and characterization for each study are summarised.

Table 2.1 General characteristics of the participants and study designs included in the present dissertation.

Study	n	Age (years)	Sex	Design
Study 1 (Chapter IV)	332	Mean = 20.4 (SD = 4.3) range: 16 to 38 years old	36.7% females	Cross-sectional
Study 2 (Chapter V)	143	Mean = 21.1 (SD = 4.8) range: 16 to 35 years old	27.2% females	Cross-sectional
Study 3 (Chapter VI)	60	Mean = 19.5 (SD = 4.2) range: 16 to 31 years old	28.3% females	Longitudinal
Study 4 (Chapter VII)	86	Mean = 21.2 (SD = 10.6) range: 8 to 50 years old	64.0% females	Cross-sectional

Body composition measurements

Body composition assessments were conducted in the morning after an overnight fast of at least 8 hours, with participants abstaining from caffeine and alcohol and avoiding moderate to vigorous exercise for at least 12 hours.

Anthropometric measurements

Participants were weighed to the nearest 0.1 kg using a scale (Seca, Hamburg, Germany), and their stature was measured to the nearest 0.1 cm with a stadiometer (Seca, Hamburg, Germany), according to standardized procedures[6].

Dual-energy x-ray absorptiometry

Participants underwent a whole-body DXA scan using procedures recommended by the manufacturer on two different devices. For **studies 1, 2 and 3,** a Hologic Explorer-W device (software QDR for Windows version 12.4, Hologic, Waltham, USA) was used. In **study 4**, the device utilized was a Hologic Horizon Wi (Hologic APEX software version 5.6.1.3, Hologic, Waltham, MA, USA).

The DXA measurements included whole-body BMC (kg), Mo (kg), FM (kg), FM (%), FFM (kg), and LST (kg) for studies 1, 2, 3, and 4. Because BMC represents ashed bone, BMC was multiplied by 1.0436 to convert it into total bone mineral (Mo)[7]. Additionally, in **study 4**, the analysis tool was used to draw customized ROIs for each measurement across the five segments: right arm, left arm, right leg, left leg, and trunk.

Total-body water

Criterion measures of TBW were conducted in **study 1** to be used in a 6C model and in **studies 2 and 3** to be used in a 4C model. Criterion TBW measurement was conducted through deuterium dilution, utilizing a stable gas isotope ratio mass spectrometer (Hydra 20-20, PDZ, software SerCon Control Callisto v40.0.11, Europa Scientific, UK). Following a 12-h fasting period, a urine sample was collected, and a deuterium oxide solution dose (^2H_2O) equivalent to 0.1 g/kg of BW was administered. After a 4-hour equilibration period, a second urine sample was obtained.

Bioelectrical impedance spectroscopy (BIS; model 4000B; Xitron Technologies, San Diego, CA) was used as an alternative method to deuterium dilution to determine TBW in **studies 2 and 3**. Additionally, in **Study 2,** using the

aforementioned device, whole-body R at 50 kHz was used to estimate TBW using the equations of Matias et al.[8] and Sun et al.[9].

Body Volume

BV was assessed by ADP (BOD POD® model 2007A version 5.4.3, software BOD POD Body Composition System, COSMED, Albano Laziale, Italy) in **study 1** (to serve as the criterion to develop and validate a DXA-derived BV equation and also to calculate the density of LST using a 6C model to estimate total body protein) and in **studies 2 and 3** (to be incorporated in a 4C model).

Criterion in vivo multicompartment model

A criterion 4-compartment model was used in **studies 2 and 3** to assess FM, based on the equation described by Wang et al. (2002)[10]:

$$\text{FM (kg)} = 2.748 \times BV - 0.699 \times TBW + 1.129 \times Mo - 2.051 \times BW$$

Where BV is body volume (L) from ADP, TBW is total body water (kg) from deuterium dilution, Mo is bone mineral (kg) from DXA, and BW is body mass (kg).

The propagation measurement error associated with the measurement of FM from the 4C model was estimated by assuming an average body composition for each study sample (BV, TBW, Mo, and BW) and the measurement precision of each method[11]. The calculations are described in detail in studies 2 and 3 (chapters V and VI).

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Chapter IV – Study 1

Novel comparative analysis and equation development for body volume estimation using dual-energy x-ray absorptiometry in athletes

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ARTICLE

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Novel comparative analysis and equation development for body volume estimation using dual-energy x-ray absorptiometry in athletes

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BACKGROUND/OBJECTIVES: Dual-energy x-ray absorptiometry (DXA) has been suggested as an alternative method for estimating body volume (BV), a component of the reference four-compartment body composition model. This study reports the development and validation of DXA-BV_{Silva}, a novel DXA-derived BV equation. An additional aim was to develop an estimate of lean soft tissue density (D_{LST}), a key variable in the theory-based approach, by estimating total body protein with a six-compartment model.
METHODS: A sample of 332 athletes (36.7% females) from several sports were randomly assigned to either the development ($n = 232$) or cross-validation group ($n = 100$). DXA-BV_{Silva} was developed via linear regression of DXA-measured fat, lean soft tissue, and bone mineral mass against BV measured with air displacement plethysmography (ADP). A D_{LST} estimate of 1.064 kg/L (SD = 0.006) was obtained from a subset of the development sample comprising 201 athletes (36.3% females) with available measurements of total-body water by deuterium dilution, bone mineral by DXA and BV by ADP, enabling total-body protein determination from a six-compartment model.
RESULTS: DXA-BV_{Silva} provided the closest BV estimation (mean difference = 0.05 L, SD = 0.46 L; ES [95% CI] = 0.11 [−0.08; 0.31]) and the 95% limits of agreement (−0.86 to 0.96 L) were narrower than existing empirical equations.
CONCLUSION: We conducted the first comparative analysis of both empirical and theoretical BV estimation methods using DXA, demonstrating the validity of DXA-BV_{Silva} for BV estimation in athletes, presenting a robust alternative to ADP, with potential applications in multicomponent body composition models.

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INTRODUCTION

Traditional body composition (BC) assessment often relies on a two-compartment (2 C) model, distinguishing fat and fat-free mass (FM and FFM, respectively) [1]. These models assume a constant FFM composition, neglecting variations in total body water (TBW), protein mass, and bone mineral content (BMC), and rely on a specific density value for FFM [1]. Athletes, however, often deviate from this assumed constant [2]. A more accurate BC assessment can be achieved through multi-compartmental models, such as the four-compartment (4 C) model, accounting for TBW and BMC variations [1].

Recently, several studies [3–5] have introduced a novel method for quantifying BV using DXA. This simplifies the development of multicompartment models by removing the requirement for hydrostatic weighing or air displacement plethysmography (ADP), enhancing accessibility for research and field settings [6]. This novel empirical approach is detailed in the review by Heymsfield and colleagues [6]. However, athletes who typically have higher bone mineral density, greater FFM, and lower FM levels than the general population [7, 8] should be considered a unique population. Yet, no DXA-derived BV equations have been specifically developed and cross-validated in an athletic sample.

Additionally, Heymsfield et al. [6] discussed an alternative method for estimating BV, which aligns with the longstanding theoretical basis of multicomponent body density models [6]. This theory-based approach can be described as follows:

$$\mathrm{DXA-BV_{3C}(L)} = \left(\frac{FM}{0.9007}\right) + \left(\frac{LST}{DLST}\right) + \left(\frac{Mo}{2.982}\right) \quad (1)$$

where 0.9007 kg/L represents the density of FM and 2.982 kg/L represents the density of bone mineral (Mo) [7]. In contrast to FM and Mo, lean soft tissue (LST) constitutes a diverse component encompassing various constituents: TBW, soft tissue minerals (Ms), protein, and small amounts of glycogen (Gly). Heymsfield and colleagues [6] have estimated different but similar values for the density of LST, suggesting a provisional value of 1.064 kg/L. However, none of the samples used in its derivation involved

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athletes. To calculate the density of lean soft tissue (D_{LST}), it is essential to measure total-body protein (TBPro), while other constituents can be estimated. A six-compartment (6 C) model, incorporating measurements of body mass, BV, BMC, TBW, and assumptions for Ms and Gly, can be employed for TBPro estimation [8, 9].

This study aims (i) to develop and validate a DXA-derived BV equation (DXA_{Silva}) in athletes utilizing ADP as a criterion, (ii) to calculate D_{LST} using a 6 C model to estimate TBPro, and (iii) to compare our newly generated equation, derived through regression modeling, to the theory-based approach to obtain DXA-derived BV.

METHODS

Participants

Data for this analysis were derived from previous studies conducted by Quitério et al. [10] on Portuguese young athletes (≤ 18 years old, data collected between January 2003 and December 2006) at national and international levels, and Hetherington-Rauth et al. [11], [December 2012 to March 2013] [11], Sardinha et al. (2020, [December 2012 to March 2013]) [12] and Francisco et al. (2024, [May 2022 to December 2023]) [13] on adult athletes (≥18 years old) with similar competition backgrounds. Further details regarding the sample, inclusion and exclusion criteria can be found in the respective papers [10, 11, 13].

For the current study, participants had to (i) undergo both ADP and DXA evaluations and (ii) be 16 years or older. The final sample consisted of 332 participants (36.7% females) spanning various sports: basketball ($n = 49$), swimming ($n = 42$), rugby ($n = 42$), combat sports (judo, taekwondo, karate, and boxing) ($n = 42$), athletics ($n = 34$), soccer ($n = 30$), triathlon ($n = 22$), gymnastics (acrobatic, group, rhythmic, and trampoline) ($n = 18$), volleyball ($n = 10$), surfing ($n = 7$), handball ($n = 5$), tennis ($n = 5$), hockey ($n = 4$), pentathlon ($n = 3$), CrossFit ($n = 2$), sailing ($n = 2$), ballroom dance ($n = 2$), fencing, rowing, mountain biking, marathon running, sport climbing, korfball, futsal, golf, motorcycling, paddle, water polo, powerlifting, and trail running ($n = 1$ each).

The studies were approved by the Ethics Committee of the Faculty of Human Kinetics, University of Lisbon (Lisbon, Portugal) and were conducted according to the declaration of Helsinki for human studies from the World Medical Association [14]. Informed consent was obtained from all participants.

Body composition measurements

BC assessments were conducted in the morning following an 8–10 h fasting period (both food and fluid), avoiding moderate to vigorous exercise in the previous 12 h.

Anthropometric measurements. Body weight (BW) was measured to the nearest 0.01 kg using an electronic scale (SECA model 770, Hamburg, Germany), and stature was measured to the closest 0.1 cm with a stadiometer (Seca, Hamburg, Germany). Based on test-retest performed in 10 athletes, the CV value in our laboratory for measuring BM is 0.07%.

Dual-energy X-ray absorptiometry (DXA). To estimate FM (kg), LST (kg) and BMC (kg), the DXA technique (Hologic Explorer-W, fan-beam densitometer, software QDR for Windows version 12.4, Waltham, USA) was used with a whole-body scan, as described elsewhere [15]. Because BMC represents ashed bone, BMC was multiplied by 1.0436 to convert it into total bone mineral (Mo) [16]. This software version does not utilize the National Health and Nutrition Examination Survey (NHANES) correction factor [17]. Scan positioning, acquisition, and analysis were standardized, followed the manufacturer's guidelines. Reflection scanning techniques were used for larger participants who could not fit within the DXA scanning dimensions [18]. For participants taller than the scan area body composition was assessed using the sum of two scans (head and trunk plus limbs) [19]. The coefficient of variation (CV) values in our laboratory for measuring FM, FFM and BMC are 1.7%, 0.8% and 1.3%, respectively [20].

Air displacement plethysmography (ADP). BV was assessed by ADP (BOD POD® model 2007A version 5.4.3, software BOD POD Body Composition System, COSMED, Albano Laziale, Italy). Detailed methodology is described elsewhere [21, 22]. The scale attached with the device was calibrated with two standardized weight blocks (each of 10.000 kg). The measurement of body volumes started with a standard two-point calibration process: first, with the chamber empty to establish baseline and then with a calibration cylinder (of 50.264 L) to establish range. Subsequently, the subject's body volume was measured in the testing chamber. BV was computed with measurements of raw BV (BVraw) corrected for thoracic gas volume (Vtg) and a surface area artifact. Vtg was measured using a standard pulmonary plethysmography. If the panting maneuver was not successfully obtained, a predicted Vtg was alternatively used using Crapo et al. [23] models. BV was calculated using the formula described by Dempster and Aitkens [21]:

$$\mathrm{BV(L)} = BVraw + 0.40Vtg - SAA \quad (2)$$

where BV is the body volume in litres (L), BVraw is the raw body volume in L, Vtg is the thoracic gas volume in L and SAA is the surface area artefact, a correction factor for the body surface area using Dubois and Dubois equation [24]. The room temperature was maintained at approximately 23 °C throughout the process. The CV value in our laboratory for measuring BV is 0.4% [20].

Total-body water (TBW). TBW measurement was conducted through deuterium dilution, utilizing a stable gas isotope ratio mass spectrometer (Hydra 20-20, PDZ, software SerCon Control Callisto v40.0.11, Europa Scientific, UK). Following a 12-h fasting period, a urine sample was collected, and a deuterium oxide solution dose (^2H_2O) equivalent to 0.1 g/kg of BW was administered. After a 4-h equilibration period, a second urine sample was obtained. A detailed method description can be found elsewhere [25]. Our laboratory's CV for measuring TBW is 0.3% [20].

DXA-derived total body volume

Empirical approach. To calculate body volume using DXA, a prediction equation introduced by Wilson et al. [3] was employed:

$$\mathrm{DXA - BV(L)} = \upsilon FM \times FM + \upsilon LST \times LST + \upsilon BMC \times BMC + \upsilon Residual \quad (3)$$

The υ represents volumetric densities equivalent to 1/D. Consequently, Eq. 3 can be expressed as:

$$\mathrm{DXA - BV(L)} = \left(\frac{FM}{D\,FAT}\right) + \left(\frac{LST}{D\,LST}\right) + \left(\frac{BMC}{D\,BMC}\right) + Residual\ volume \quad (4)$$

The residual term accounts for the volume not captured by the DXA method.

DXA-derived BV was also determined with the equations from Wilson et al. [3] and Smith-Ryan et al. [4].

$$\mathrm{DXA - BV_{Wilson}(L)} = \left(\frac{FM}{0.88}\right) + \left(\frac{LST}{1.05}\right) + \left(\frac{BMC}{4.85}\right) + 0.01 \quad (5)$$

$$\mathrm{DXA - BV_{Smith-Ryan}(L)} = \left(\frac{FM}{0.84}\right) + \left(\frac{LST}{1.03}\right) + \left(\frac{BMC}{11.63}\right) - 3.12 \quad (6)$$

Even though both equations were derived using Hologic DXA models, there were differences in the analysis methods used. Wilson et al. [3] utilized the NHANES correction factor [17]. Consequently, the variables in this equation underwent adjustment beforehand with this correction factor during data processing (corrected LST was obtained by multiplying the DXA-derived LST by 0.946, and corrected FM was calculated by subtracting the sum of corrected LST and BMC from total DXA mass [17]).

Theory-based approach. DXA-derived BV estimation was also estimated following a theory-based approach proposed by Heymsfield et al. (Eq. 1) [6].

To determine D_{LST}, TBPro was calculated using a 6 C model [8], where BW is expressed as the sum of FM, TBW, TBPro, Mo, Ms, and Gly:

$$\mathrm{BW} = FM + TBW + TBPro + Mo + Ms + Gly \quad (7)$$

Consequently, the 6-C TBPro model is formulated as:

$$TBPro = BW - FM - TBW - Mo - Ms - Gly \quad (8)$$

All components are currently either quantifiable or computable. FM can be measured using a 4 C model [26] (FM = 2.748 × BV - 0.699 × TBW + 1.129 × Mo - 2.051 × BW), where BV (L) is determined by ADP, TBW

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(kg) is measured by deuterium dilution, Mo (kg) is obtained from BMC measured by DXA (Mo = 1.0436 × BMC) and BW is in kg. Additionally, Ms (kg) can be calculated from total body water (kg) as Ms = 0.0129 × TBW [26], and Gly (kg) can be calculated from total body protein (kg) as Gly = 0.044 × TBPro [27].

Substituting FM, Ms and Gly into Eq. 8 allows us to rewrite it as:

$$TBPro = BW - (2.748 \times BV - 0.699 \times TBW + 1.129 \times Mo - 2.051 \times BW) - TBW - Mo - 0.0129 \times TBW - 0.044 \times TBPro \quad (9)$$

Rearranging to isolate TBPro gives:

$$1.044 \times TBPro = 3.051 \times BW - 0.314 \times TBW - 2.129 \times Mo - 2.748 \times BV \quad (10)$$

Finally, solving for TBPro, the 6 C TBPro model (Eq. 11) can be formulated and utilized as [8]:

$$TBPro = 2.922 \times BW - 0.301 \times TBW - 2.039 \times Mo - 2.632 \times BV \quad (11)$$

From this equation, LST can be defined as the sum of TBW, Ms, TBPro and Gly [7]:

$$LST = TBW + Ms + TBPro + Gly \quad (12)$$

Dividing by the respective densities:

$$\frac{LST}{D\,LST} = \frac{TBW}{0.9937} + \frac{Ms}{3.317} + \frac{TBPro}{1.34} + \frac{Gly}{1.52} \quad (13)$$

where 0.9937, 3.317, 1.34 and 1.52 kg/L are the established densities at body temperature of TBW, Ms, TBPro and Gly, respectively [7].

Equation 13 can now be rearranged to solve for D_{LST}, replacing the other already known or estimated constituents:

$$DLST = \frac{LST}{\frac{TBW}{0.9937} + \frac{Ms}{3.317} + \frac{TBPro}{1.34} + \frac{Gly}{1.52}} \quad (14)$$

Propagation of measurement error

In the present study, the error associated with the determination of TBProt can be estimated by assuming average BC of the whole sample (BV, TBW, Mo and BW) and measurement precision as stated in the methods section. Accordingly:

$$TBPro\,\upsilon^2 = (2.922 \times 70.4 \times 0.0007)^2 + (0.301 \times 44.6 \times 0.003)^2 + (2.039 \times 2.9 \times 0.013)^2 + (2.632 \times 66.04 \times 0.004)^2 = 0.510\,(\upsilon = 0.714\,kg)$$

Based on this calculation, the precision was nearly 0.7 kg for TBPro determination.

Statistical analysis

IBM SPSS Statistics® (IBM, Chicago, Illinois, USA) version 29.0 for Windows® was used for data analysis. Statistical significance was set at 5% (two-sided). Data were reported as mean (standard deviation [SD]). We evaluated normality by skewness and kurtosis. The values of skewness and kurtosis of the DXA scan (FM, LST and BMC) variables and ADP BV were within the acceptable ranges of |skewness| < 3 and |kurtosis| < 8 [28].

Before the regression analysis, descriptive statistics were calculated on all participants for data screening and cleaning. Two participants were excluded due to inconsistent values. Eighteen outliers were removed due to a Cook's distance greater than 4 / (n − k − 1), where n is the sample size and k is the number of explanatory variables in the model [29].

Individuals were allocated into the development group (70%) or the cross-validation group (30%) using stratified randomization to ensure a balanced sex ratio. The development group ($n = 232$) and the cross-validation group ($n = 100$) did not significantly differ regarding sex, age, weight, height, BMI, BMC, FM, LST and ADP BV.

The coefficients in Eq. 3 were determined through multiple linear regression using the DXA and ADP measurements obtained from the development sample. The BV from ADP was used as dependent variable, and FM, LST and BMC from DXA as independent variables.

The new coefficients were then applied to calculate DXA-derived BV in the cross-validation sample. This aimed to assess the accuracy of the new equation for estimating BV in athletes. Paired samples' *t*-tests were used to determine whether BV assessments from the newly derived and previous equations ($DXA\text{-}BV_{Wilson}$ and $DXA\text{-}BV_{Smith\text{-}Ryan}$) differed from the criterion (ADP-BV). Effect sizes were measured using Cohen's d, classified according to Hopkin's scale [30]: [0; 0.2[= trivial, [0.2; 0.6[= small, [0.6; 1.2[= moderate, [1.2; 2.0[= large, ≥ 2.0 = very large. Pearson's correlation coefficients (r) and mean differences (MD), obtained by subtracting the values estimated from DXA from the value obtained by ADP-BV, were also calculated.

Furthermore, we evaluated the agreement between DXA-derived BV estimates and ADP-BV using the Bland-Altman method [31] using GraphPad Prism® software version 10.2.2.

RESULTS

Table 1 presents the participants' characteristics for the development and cross-validation samples. There were no significant differences between groups. Subjects exhibited a weight range of 41.3 kg to 114.2 kg, with an average body fat percentage measured by DXA of 17.6% (SD = 6.6), ranging from 6.6% to 38.1%. Participant height ranged from 146.0 cm to 205.0 cm, and mean age was 20.4 years (SD = 4.3), ranging from 16 to 38 years.

Empirical body volume equation

The coefficients associated with Eq. 3 were: υFM = 1.128 ($p < 0.001$), υLST = 0.953 ($p < 0.001$), υBMC = 0.128 ($p = 0.227$), and υresidual = −0.126 ($p = 0.481$). These coefficients indicate average densities for FM, LST, and BMC of 0.89, 1.05, and 7.81 kg/L, respectively. The residual coefficient corresponds to a volume of −0.126 L, representing the portion not accounted for by the FM, LST, and BMC components.

$$DXA - BV_{Silva}(L) = \left(\frac{FM}{0.89}\right) + \left(\frac{LST}{1.05}\right) + \left(\frac{BMC}{7.81}\right) - 0.126$$

The accuracy of the developed model can be observed by the high coefficient of determination ($R^2 = 0.998$) and low standard error of estimation (SEE = 0.475 L). Additional details, including the unstandardized (B) and standardized coefficients (β), variance inflation factors (VIF), and p-values for each variable, are presented in Supplementary Table 1.

D_{LST} estimation for the theory-based body volume equation

To apply the theory-based body volume equation described in Eq. 1, it was necessary to derive a D_{LST} estimation. A subset of the development sample comprising 201 athletes (36.3% females) contributed with data for D_{LST} estimation (i.e., available measurements of TBW by deuterium dilution, Mo through DXA, and BV by ADP, enabling TBPro determination from a 6 C model). For this analysis, participants with FFM hydration (TBW/FFM) values outside the range reported in 9 adult human cadavers [32] (0.686 to 0.808) were excluded. The subjects had a mean age of 21.0 (SD = 4.7) years, with average weight and height of 71.1 (SD = 12.6) kg and 175.4 (SD = 9.9) cm, respectively. Additionally, the mean %FM and FFM calculated using the 4 C model were 14.1% (SD = 7.2) and 61.2 kg (SD = 12.6), respectively.

Among males, the average TBPro was 14.00 kg (SD = 2.08), while for females it was 10.10 kg (SD = 1.53). For the combined sample, TBPro averaged 12.58 kg (SD = 2.67). Using Eq. 14, the D_{LST} in this subset of 201 athletes from the development sample was calculated to be 1.064 kg/L (SD = 0.006). Comparable findings were observed when D_{LST} was computed separately per sex, with no significant difference between males and females ($p = 0.127$; ES [95% CI] = 0.23 [−0.06; 0.51]).

Athletes were categorized into weight-sensitive (WS, $n = 71$) and non-weight-sensitive sports (nWS, $n = 130$) based on Ackland et al.'s (2012) [33] criteria, with D_{LST} showing no significant

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Table 1. Participants' characteristics and body composition for development and cross-validation samples.

	Development sample						Cross-validation sample					
	Whole sample ($n = 232$)		Female ($n = 85$)		Male ($n = 147$)		Whole sample ($n = 100$)		Female ($n = 37$)		Male ($n = 63$)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Age (years)	20.6	4.6	20.6	4.4	20.6	4.7	19.9	3.5	19.7	3.0	20.1	3.8
Weight (kg)	70.4	12.7	60.9	8.6	75.9	11.4	69.3	12.0	60.0	8.8	74.7	10.1
Height (cm)	175.1	9.9	167.3	8.7	179.6	7.5	173.8	9.9	165.9	7.8	178.4	7.8
BMI (kg/m^2)	22.8	2.7	21.7	2.0	23.5	2.9	22.8	2.5	21.7	2.4	23.4	2.4
BMC (kg)	2.8	0.6	2.4	0.4	3.0	0.5	2.8	0.6	2.4	0.4	3.0	0.5
Fat mass (kg)	12.2	4.8	14.4	4.3	10.9	4.6	11.9	4.6	14.5	4.8	10.3	3.8
Fat mass (%)	17.7	6.5	23.7	5.3	14.2	4.2	17.6	6.7	24.1	5.3	13.8	4.0
LST (kg)	54.6	11.1	43.4	5.9	61.1	7.7	53.8	11.1	42.4	5.3	60.4	7.5
FFMI (kg/m^2)	18.56	2.44	16.32	1.34	19.86	1.94	18.54	2.50	16.23	1.46	19.90	1.91
ADP BV (L)	66.04	11.91	57.82	8.41	70.8	11.0	64.81	11.14	56.93	8.77	69.44	9.73

SD standard deviation, *BMI* body mass index, *BMC* bone mineral content, *LST* lean soft tissue, *ADP* air displacement plethysmography, *FFMI* fat-free mass index, *BV* body volume.

differences between WS and nWS sports ($p = 0.549$; ES [95% CI] = 0.09 [−0.20; 0.38]). Subdividing by sex also did not show significant differences (males: $p = 0.687$; ES [95% CI] = 0.08 [−0.30; 0.45]; females: $p = 0.849$; ES [95% CI] = 0.04 [−0.42; 0.51]).

Cross-validation of the new DXA-derived body volume equation and comparison with other empirical approach equations

The comparison between empirical DXA-derived BV estimates and ADP-BV is presented in Table 2. DXA-BV$_{Silva}$ did not differ significantly ($p = 0.261$; ES [95% CI] = 0.11 [−0.08; 0.31]; MD = 0.05 L, SD = 0.46) compared to the criterion. In contrast, DXA-BV$_{Wilson}$ and DXA-BV$_{Smith\text{-}Ryan}$ estimated significantly higher and lower BV ($p < 0.001$; MD = 0.97 L, SD = 0.51, and $p < 0.001$; MD = −1.38 L, SD = 0.56) with large and very large effect sizes (ES [95% CI] = 0.31 [0.11; 0.51] and ES [95% CI] = 2.47 [2.07; 2.86]), respectively.

The Bland-Altman plots (Fig. 1) illustrate the agreement between the BV values. The 95% limits of agreement ranged from −0.86 to 0.96 L for DXA-BV$_{Silva}$, −1.96 to 0.03 L for DXA-BV$_{Wilson}$, and 0.28 to 2.47 L for DXA-BV$_{Smith\text{-}Ryan}$. A weak proportional bias was found for DXA-BV$_{Smith\text{-}Ryan}$ ($r = -0.393$, $p < 0.001$).

Cross-validation of the theory-based approach equations

The comparison between DXA-derived BV estimates from the theory-based approach (Eq. 1) and ADP-BV is presented in Table 2. We investigated the provisional D_{LST} value of 1.064 kg/L [6] which aligns with our finding in 201 athletes, and examined the impact of using BMC or Mo. A primary finding indicates that incorporating Mo instead of BMC in Eq. 1 slightly improves the predictive capability. The Bland-Altman plots in Fig. 2 illustrate the agreement between the prediction equations using Heymsfield's and our new D_{LST} value (DXA-BV$_{Heymsfield_1.064_BMC}$, DXA-BV$_{Heymsfield_1.064_Mo}$) compared to ADP. The 95% limits of agreement ranged from −0.73 to 1.09 L for DXA-BV$_{Heymsfield_1.064_BMC}$ and −0.76 to 1.05 L for DXA-BV$_{Heymsfield_1.064_Mo}$. A very weak proportional bias was found for DXA-BV $_{Heymsfield_1.064_BMC}$ ($r = 0.200$, $p = 0.045$).

Overall DXA-derived BV equations

Overall, the DXA-BV$_{Silva}$ provided the closest BV estimation (MD = 0.05, SD = 0.46; $p = 0.261$; ES [95% CI] = 0.11 [−0.08; 0.31] L). Furthermore, it displayed the lowest root mean squared error (RMSE) of 0.465 L, contrasting with the DXA-BV$_{Smith\text{-}Ryan}$ equation, which exhibited the highest RMSE (1.483 L).

The validation of DXA-derived BV estimates for females and males can be consulted in Table 3. For females, only DXA-BV$_{Silva}$ (mean = 56.83 L, SD = 8.77) did not differ significantly ($p = 0.079$; MD = 0.11 L, SD = 0.36) compared to the criterion. For males, DXA-BV$_{Silva}$ (mean = 69.42 L, SD = 9.67) and the theory-based approach, using Mo, proposed by Heymsfield et al. [6] (mean = 69.31 L, SD = 9.62) did not differ significantly ($p = 0.745$; MD = 0.02 L and $p = 0.057$; MD = 0.13 L, respectively) compared to the ADP.

DISCUSSION

In this study, we formulated the DXA-BV$_{Silva}$ prediction model by integrating DXA coefficients involving FM, LST, and BMC. Our model demonstrated reliable prediction accuracy for BV when compared to the conventional ADP method. Furthermore, we investigated the validity of a theory-based volume estimation approach in athletes and derived new data on D_{LST}, contributing to a more comprehensive understanding of BC within athletic contexts.

DXA-BV$_{Silva}$ exhibited negligible mean differences with ADP-BV in a diverse group of athletes. The Bland-Altman plot revealed minor individual differences with a small bias (0.05 L), and the 95% confidence intervals found were narrower than those reported by other authors [4]. The absence of proportional bias is particularly pertinent when practical constraints render ADP or UWW impractical or unavailable.

Since type I methods (as regression-derived equations for predicting body composition based on measurable properties [34]) are population- and condition-specific, they must be revalidated when applied to different populations or conditions [34]. This study found significant mean differences between pre-existing DXA-derived BV equations and the criterion measure. The equation by Smith-Ryan et al. [4] yielded considerably lower BV values with wide 95% limits of agreement, indicating substantial measurement discrepancies and significant proportional bias. Additionally, Wilson's Eq. (3) significantly overestimated BV but was closer to the criterion measure, which was expected given Wilson's sample characteristics more closely resemble those of athletes. Wilson's study [3] involved 25 participants with an average age of 37.6 years (SD = 15.5) and a BMI of approximately

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Table 2. Validation of DXA-derived empirical and theory-based BV estimates in the cross-validation sample.

	Method	Mean (SD)	MD (SD)	*p*	r (p)	ES	Bland-Altman		r (p)
						Cohen' d [95% Confidence Interval]	95% LOA Lower	Upper	
	Whole sample ($n = 100$)								
	ADP-BV	64.81 (11.14)							
Empirical body volume equations	**Cross-validation**								
	$DXA\text{-}BV_{Silva}$	64.76 (11.09)	0.05 (0.46)	0.261	0.999 (<0.001)	0.11 [−0.08; 0.31]	−0.86	0.96	0.099 (0.325)
	Validation								
	$DXA\text{-}BV_{Wilson}$	65.78 (11.22)	−0.97 (0.51)	<0.001	0.999 (<0.001)	−1.90 [-2.23; −1.57]	−1.96	0.03	−0.163 (0.106)
	$DXA\text{-}BV_{Smith\text{-}Ryan}$	63.44 (11.36)	1.38 (0.56)	<0.001	0.999 (<0.001)	2.47 [2.07; 2.86]	0.28	2.47	−0.393 (<0.001)
Theory-based body volume equations	BV = (FM/0.9007) + (LST/**1.064**) + (**BMC**/2.982)	64.63 (11.05)	0.18 (0.46)	<0.001	0.999 (<0.001)	0.40 [0.19; 0.60]	−0.73	1.09	0.200 (0.045)
	BV = (FM/0.9007) + (LST/**1.064**)+(**Mo**/2.982)	64.66 (11.05)	0.14 (0.46)	0.003	0.999 (<0.001)	0.31 [0.11; 0.51]	−0.76	1.05	0.186 (0.064)

ADP-BV air displacement plethysmograph body volume, *BMC* bone mineral content, *BV* body volume, *ES* Effect Size, *MD* mean difference (subtraction of the value obtained by DXA-derived equations from the value estimated by ADP-BV), *Mo* bone mineral, *r* correlation coefficient, *SD* standard deviation, *95% LOA* 95% limits of agreement (MD ± 1.96 x SD of differences). A comparison of means between methods was performed using the paired samples *t*-test.

24 kg/m^2, while Smith-Ryan's study [4] had a larger sample ($n = 127$) with a similar age range but encompassing a broader BMI spectrum (mean = 31.4 kg/m^2, SD = 5.5). This study confirms that empirical DXA-BV models are inherently population-specific, particularly considering athletes who exhibit distinct characteristics such as higher bone mineral density, greater FFM, and generally lower body fat levels [35, 36].

Our investigation not only scrutinized the appropriateness of DXA-derived BV estimates in this athletic cohort but also proposed a novel equation specifically tailored for BV estimation in athletes. This involved rigorous development and cross-validation utilizing state-of-the-art methods as the criterion, thereby enhancing the generalizability of this approach. Furthermore, we conducted a novel comparative analysis of both empirical and theoretical methods for estimating BV from DXA. Specifically, we evaluated the validity of a theory-based volume estimation approach in athletes and, for the first time to our knowledge, estimated a D_{LST} value from an athletic sample. Although derived from non-athletic samples, the provisional value of 1.064 kg/L [6] is closely aligned with our density estimation. Moreover, integrating Mo instead of BMC in Eq. 1 slightly enhances the model's predictive capacity.

The foundational assumptions of theory-based DXA volume models rely on the accuracy and constancy of DXA FM, LST, and osseous mineral mass estimates, supported by well-established and historically validated densities [6, 7], except for LST, which is newly derived in this study. Our findings revealed a density of 1.064 kg/L in a sub-sample characterized by diminished FFM hydration (TBW/FFM = 72.7%) compared to the average values derived from three male cadavers [37]. Heymsfield et al. [6] proposed a provisional value based on four study samples, yielding estimated values ranging from 1.062 to 1.068 kg/L, with a mean density of 1.064 kg/L. Similarly, we observed a mean density of 1.064 kg/L (range: 1.041 to 1.077). The observed variations in D_{LST} values underscore the susceptibility of its constituents, most probably TBProt and TBW, to induce relative changes capable of perturbing the proportions within the sample datasets used to derive these values. The variability in D_{LST} raises hypotheses about potential influencing factors, including hydration status, individual traits, or different sports disciplines. A regression analysis between LST density and FFM hydration initially indicated that FFM hydration explained 96.5% of LST density variability. Recognizing that FFM from the 4 C model includes TBW, we analyzed FFM measured by DXA, finding it explained 57.4% of D_{LST} variability, with lower FFM hydration strongly associated with higher LST density ($r = -0.757$, $p < 0.001$). Therefore, lower TBW content results in higher overall LST density. This highlights the potential impact of slight deviations, possibly attributed to challenges in maintaining strict control over athletes' hydration status [38]. Given that TBW is the LST constituent with the lowest density, any errors arising from underestimating this value would result in a higher D_{LST} value. Similarly observed by Bennett et al. [39] in a sample of adults and collegiate-aged student-athletes, our observed average hydration fraction of FFM was lower than both the established constant of 73.2% from studies in mammals and human cadavers [32]. Sagayama et al. [40], also reported reduced FFM hydration levels in athletes. This underscores the significance of a multicompartment assessment approach over traditional two-compartment models reliant on assumed hydration factors, especially in specific populations such as athletes.

The main strength of this study is that it included a large sample of athletes across several different sports. Additionally, the sample size utilized for model development was considerably larger than that of Wilson et al. ($n = 25$) [3] and Smith-Ryan et al. ($n = 127$) [4], indicating that each participant exerted less influence on the developed coefficients. Nevertheless, several limitations warrant consideration in interpreting our findings. Firstly, as the coefficients were derived from a Hologic DXA model, applying the new equation to BV estimates obtained from alternative DXA devices

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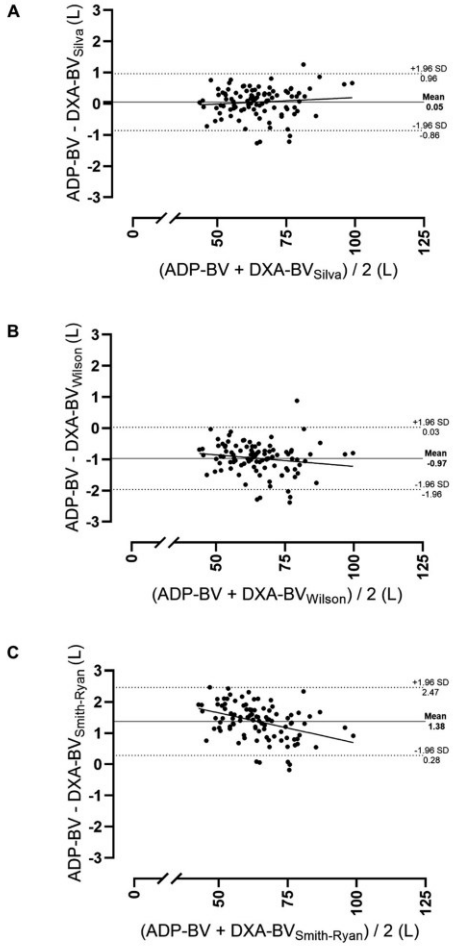

Fig. 1 Bland-Altman analysis between the prediction equations BV and ADP-BV. The solid lines represent the mean differences between the DXA-derived BV estimates (DXA-BV$_{Silva}$ (**A**). DXA-BV$_{Wilson}$ (**B**) and DXA-BV$_{Smith-Ryan}$ (**C**)) and ADP-BV. The upper and lower dashed lines represent 95% limits of agreement (± 1.96 SD). The trend lines represent the degree of association between the mean and the difference, as illustrated by Pearson's correlation coefficient (r).

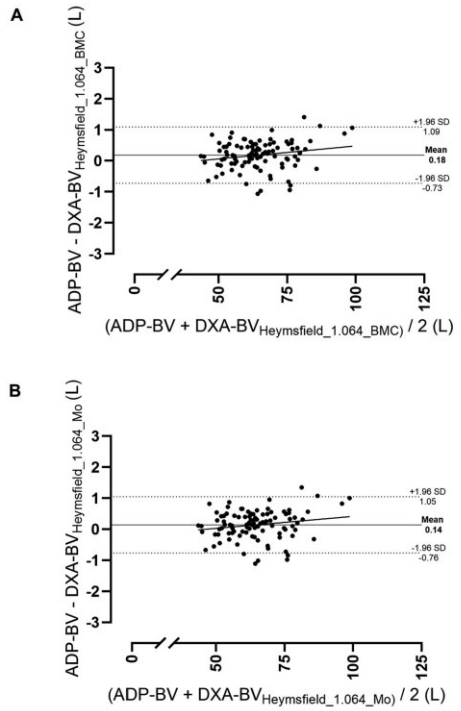

Fig. 2 Bland-Altman analysis between the prediction equations BV and ADP-BV. The solid lines represent the mean differences between the DXA-derived BV estimates (DXA-BV$_{Heymsfield_1.064_BMC}$ (**A**) and DXA-BV$_{Heymsfield_1.064_Mo}$ (**B**)) and ADP-BV. The upper and lower dashed lines represent 95% limits of agreement (±1.96 SD). The trend lines represent the degree of association between the mean and the difference, as illustrated by Pearson's correlation coefficient (r).

may result in reduced accuracy. We also acknowledge that the specified DXA software version (QDR for Windows version 12.4) corresponds to the most recent version and was used for the majority of the data collection. However, as this study includes data collected over several years in our laboratory, we recognize that different versions of the DXA software may have been utilized during this period. This variability represents a potential source of error that should be considered when interpreting the results.

Additionally, the development sample exclusively comprised athletes aged ≥ 16 years, restricting generalization. Moreover, most were below 25 years, further constraining the extrapolation of results to older athletic populations. Furthermore, calculating D_{LST} requires measuring TBPro, but traditional in vivo TBPro assessment via neutron activation analysis was not utilized; instead, a 6 C model was employed, incorporating body mass, BV, BMC, TBW measurements, and assumptions for Ms and Gly. Lastly, to enhance the robustness of the newly proposed BV equation, future investigations should encompass diverse athletic samples to validate its applicability in different age ranges and with a higher female representation across varying athletic demographics.

In conclusion, our study has contributed to the field by developing and validating a DXA-derived BV equation, expanding upon the groundwork conducted by Wilson et al. [3] and enabling BV estimation in athletes. Furthermore, to the best of our knowledge, we conducted the first comparative analysis of both empirical and theoretical approaches for estimating BV from DXA. Our results revealed that DXA-BV$_{Silva}$ provided the most accurate estimate of BV for female and male athletes. Future research should explore the validity of a rapid 4 C model to estimate FM in

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Table 3. Validation of DXA-derived BV estimates for females and males in the cross-validation sample.

					ES	Bland-Altman		
						95% LOA		*r* (p)
Method	**Mean (SD)**	**MD (SD)**	***p***	***r* (p)**	**Cohen' d [95% Confidence Interval]**	**Lower**	**Upper**	
Females ($n = 37$)								
DXA-BV$_{Silva}$	56.83 (8.77)	0.11 (0.36)	0.079	0.999 (<0.001)	0.30 [−0.03; 0.62]	−0.59	0.80	0.314 (0.058)
DXA-BV$_{Wilson}$	57.76 (8.78)	−0.82 (0.34)	<0.001	0.999 (<0.001)	−2.41 [−3.05; −1.76]	−1.49	−0.15	−0.038 (0.826)
DXA-BV$_{Smith-Ryan}$	55.49 (8.99)	1.45 (0.46)	<0.001	0.999 (<0.001)	3.13 [2.33; 3.91]	0.54	2.35	−0.476 (0.03)
BV = (FM/0.9007) + (LST/**1.064**) + (**BMC**/2.982)	56.73 (8.63)	0.21 (0.37)	0.002	0.999 (<0.001)	0.56 [0.21; 0.91]	−0.52	0.93	0.373 (0.023)
BV = (FM/0.9007) + (LST/**1.064**)+(**Mo**/2.982)	56.76 (8.64)	0.17 (0.37)	0.007	0.999 (<0.001)	0.47 [0.13; 0.81]	−0.55	0.89	0.362 (0.028)
Males ($n = 63$)								
DXA-BV$_{Silva}$	69.42 (9.67)	0.02 (0.52)	0.745	0.999 (<0.001)	0.04 [−0.21; 0.29]	−1.00	1.04	0.130 (0.310)
DXA-BV$_{Wilson}$	70.49 (9.77)	−1.05 (0.57)	<0.001	0.999 (<0.001)	−1.84 [−2.24; 1.43]	−2.17	0.07	−0.060 (0.642)
DXA-BV$_{Smith-Ryan}$	68.10 (9.96)	1.33 (0.61)	<0.001	0.999 (<0.001)	2.20 [1.74; 2.66]	0.15	2.52	−0.380 (0.002)
BV = (FM/0.9007) + (LST/**1.064**) + (**BMC**/2.982)	69.27 (9.62)	0.17 (0.51)	0.011	0.999 (<0.001)	0.33 [0.08; 0.58]	−0.84	1.18	0.228 (0.072)
BV = (FM/0.9007) + (LST/**1.064**)+(**Mo**/2.982)	69.31 (9.62)	0.13 (0.51)	0.057	0.999 (<0.001)	0.24 [−0.01; 0.49]	−0.88	1.13	0.218 (0.086)

ADP-BV air displacement plethysmograph body volume, *BMC* bone mineral content, *BV* body volume, *ES* Effect Size, *MD* mean difference (subtraction of the value obtained by DXA-derived equations from the value estimated by ADP-BV), *Mo* bone mineral, *r* correlation coefficient, *SD* standard deviation, *95% LOA* 95% limits of agreement (MD ± 1.96 x SD of differences). A comparison of means between methods was performed using the paired samples *t*-test.

athletes using the DXA-derived BV estimate, potentially eliminating the requirement for UWW or ADP.

DATA AVAILABILITY

Data described in the manuscript will be made available upon reasonable request pending application and approval to analiza@fmh.ulisboa.pt.

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AUTHOR CONTRIBUTIONS

TRS: Conceptualization, Methodology, Formal Analysis, Writing-Original draft preparation, FJ: Writing - Review & Editing, RP: Formal analysis, Writing - Review & Editing, LBS: Writing - Review & Editing, Supervision, Funding acquisition, VHT: Writing - Review & Editing, SBH: Writing - Review & Editing, Supervision, AMS: Conceptualization, Methodology, Writing - Review & Editing, Supervision, Funding Acquisition.

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COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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Supplementary Material
Novel comparative analysis and equation development for body volume estimation using dual-energy x-ray absorptiometry in athletes

Supplementary Table 1 Development of the dual-energy x-ray absorptiometry (DXA) derived body volume equation by multiple regression.

	Unstandardized Coefficient	Standardized Coefficient	p	VIF
(Constant)	- 0.126		0.481	
FM	1.128	0.456	<0.001	1.106
LST	0.953	0.888	<0.001	3.565
BMC	0.128	0.006	0.227	3.660

BMC bone mineral content. **FM** fat mass. **LST** lean soft tissue. **VIF** variance inflation factor.
Method: Enter

Chapter V – Study 2

Rapid Four-Compartment Models in Athletes Using Alternative Solutions for Body Volume and Water

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ORIGINAL ARTICLE

Rapid Four-Compartment Models in Athletes Using Alternative Solutions for Body Volume and Water

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Keywords: athletes | body composition | dual-energy-X-ray absorptiometry | multicompartment model | validation

ABSTRACT

The criterion four-compartment (4C) model combines dual-energy x-ray absorptiometry (DXA), deuterium dilution, and air displacement plethysmography (ADP), but its complexity limits practicality. This study aimed to externally validate a DXA-derived body volume (BV) equation (DXA-BV_{Silva}) and assess the accuracy of a rapid 4C model replacing deuterium dilution total body water (TBW) with bioelectrical impedance spectroscopy (BIS) alongside DXA-derived BV in athletes. A total of 143 athletes (27.3% females) validated DXA-BV, with 115 (33.9% females) assessed for FM. Criterion 4C used DXA for bone mineral content, ADP for BV, and deuterium dilution for TBW. Several rapid 4C models were tested. DXA-BV_{Silva} and DXA-$BV_{Heymsfield}$ reliably estimated BV, showing minimal mean differences and narrow 95% limits of agreement (LOA) compared to ADP. Among rapid 4C models, 4CTBW_{BIS}, 4C $BV_{Silva}TBW_{BIS}$, and 4C $BV_{Heymsfield}TBW_{BIS}$ provided the most accurate FM estimates with small, nonsignificant differences to the criterion (MD [SD]: −0.18 [1.25] kg; −0.23 [1.82] kg; 0.18 [1.87] kg, respectively) and narrow 95% LOA (−2.62 to 2.27, −3.79 to 3.33 and −3.48 to 3.83 kg, respectively) with no proportional bias. This research supports the implementation of rapid 4C models in settings where the criterion 4C model is impractical. By improving accuracy in body composition assessment, our findings have implications for sports nutrition, sports science, and academic research, offering a viable alternative to traditional 2C and 3C models (DXA).

1 | Introduction

Traditional field methods for body composition assessment commonly rely on two-compartment (2C) models, which categorize the body into fat (FM) and fat-free mass (FFM) [1]. These models assume a uniform FFM composition, disregarding variations in total body water (TBW), protein mass, and bone mineral content (BMC). They also rely on fixed constants, such as an FFM density of 1.100 g/cm^3 or an FFM/TBW ratio of 0.732 [1]. However, research has demonstrated that athletes often diverge from these assumptions [2, 3], originally derived from human cadaver analysis studies [4].

In previous studies conducted by our laboratory [5, 6], and consistent with recent findings by Bennett et al. [7], the hydration fraction of FFM in athletic samples was observed to diverge from the commonly assumed constant of 73.2%. This highlights the limitations of traditional 2C models and emphasizes the need for multicompartment assessment methods that account for both inter-individual differences [2, 3] and intra-individual variability [3] in body composition.

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Acknowledging individual variations in FFM hydration and BMC—both of which affect the assumed density of FFM (1.100 g/cm^3) in 2C models—led to the development of multicompartment models. The four-compartment (4C) model, by separately accounting for TBW and BMC, minimizes these sources of error and is considered the current reference for assessing FM [8]. The 4C model integrates measurements from dual-energy x-ray absorptiometry (DXA) for BMC assessment, deuterium dilution for TBW measurement, and air displacement plethysmography (ADP) or hydrostatic weighing for determining body volume (BV). While these methods significantly increase the accuracy and precision of body composition assessments, they are technically complex, time-intensive, costly, less accessible, and burdensome for participants [9], limiting their practicality for routine use.

To address these limitations, a simplified version of the 4C model was developed [10, 11] using bioelectrical impedance analysis (BIA) for TBW estimation and DXA for BV determination [12, 13]. This rapid 4C model has a shorter time burden for participants, offering a significant advantage over the conventional 4C model, particularly for athletes with tight schedules and intense training regimens.

Previous studies have assessed the validity of rapid 4C models in physically active adults [10, 14], individuals with overweight or obesity [15], and adults with varying body mass index (BMI) and waist circumference profiles [16]. There is no athlete-specific rapid 4C model and previous studies have relied on DXA-derived BV equations developed in nonathletic populations. These regression-based equations are inherently population-specific and require revalidation when applied to different groups [17]. Building on Wilson et al.'s work [12], our team has recently developed a DXA-derived BV equation tailored for athletes [18], which demonstrated the highest accuracy for both female and male athletes compared to existing equations. This advancement now enables us to evaluate the validity of the rapid 4C model in athletic populations.

Indeed, a recent systematic review by Serafini et al. highlighted that most anthropometric equations for athletes were developed using DXA as a reference, with only one study using a 4C model [19]. This emphasizes another potential application of rapid 4C models as an alternative criterion for body composition assessment in settings where the full implementation of the true 4C model is not feasible.

Therefore, the present study aims (i) to externally validate our previously developed DXA-derived BV equation (DXA-BV$_{Silva}$) in an independent sample of athletes using ADP as the criterion and (ii) to assess the accuracy of a rapid 4C model to estimate FM by replacing deuterium dilution TBW measurements with BIA alongside DXA-derived BV estimates.

2 | Methods

2.1 | Participants

This study utilized data previously collected within two research projects by Silva et al. (2010) [20] and Silva et al. (2017) [21]. The former analyzed a sample of national top-level judo athletes, while the latter recruited athletes competing in national and international championships across several sports. Detailed information on the sample, as well as inclusion and exclusion criteria, can be found in the respective publications.

For this analysis, participants with FFM hydration (TBW/FFM) values outside the range reported [22] in nine adult human cadavers (0.686 to 0.808) were excluded ($n = 28$). The final sample consisted of 143 participants (27.3% females) from several sports: 31 from basketball, 28 from judo, 23 from volleyball, 21 from triathlon, 19 from swimming, 17 from handball, three from pentathlon, and one from tennis. A subset of the total sample comprising 115 athletes (33.9% females) provided the necessary data to assess the accuracy of the rapid 4C models.

The Ethics Committee of the Faculty of Human Kinetics, University of Lisbon (Lisbon, Portugal) approved the studies. They were conducted according to the declaration of Helsinki for human studies from the World Medical Association [23].

2.2 | Body Composition Measurements

Body composition assessments were conducted in the morning after a 12-h fast, with participants abstaining from caffeine and alcohol for the previous 24 h and avoiding vigorous exercise for at least 15 h.

2.2.1 | Anthropometric Measurements

Body weight (BW) was measured to the nearest 0.01 kg on an electronic scale connected to the ADP computer, with participants barefoot and wearing a bathing suit. Stature was measured to the closest 0.1 cm with a stadiometer (Seca, Hamburg, Germany). The coefficient of variation (CV) for BW, based on test–retest using ten participants, is 0.07%.

2.2.2 | Dual-Energy X-Ray Absorptiometry (DXA)

FM (kg), FFM (kg) and BMC (kg) were estimated using the DXA technique (Hologic Explorer-W, Waltman, MA, USA; software QDR for Windows version 12.4) with a whole-body scan, as described elsewhere [24]. The equipment measures the attenuation of X-rays pulsed between 70 and 140 kV synchronously with the line frequency for each pixel. Following the protocol for DXA described by the manufacturer, a step phantom with six fields of acrylic and aluminum of varying thickness and known absorptive properties was scanned to serve as an external standard for analyzing different tissue components. Scan positioning, acquisition, and analysis were standardized and followed the manufacturer's guidelines. This software version does not utilize the National Health and Nutrition Examination Survey (NHANES) correction factor [25]. Reflection scanning techniques were used for larger participants who could not fit within the DXA scanning dimensions [26]. For participants taller than the scan area, body composition was assessed using the sum of two scans (head and trunk plus limbs) [27]. Furthermore, DXA allows dividing FFM into two components: lean soft tissue (LST) and BMC. Because BMC represents ashed bone, BMC was multiplied by

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1.0436 to convert it into total bone mineral (Mo). The CV values in our laboratory for measuring BW, FM, FFM, and BMC are 1.7%, 0.8%, and 1.3%, respectively [5].

2.2.3 | DXA-Derived Total BV

DXA-derived BV was determined with the following equations from Silva et al. (2025) [18] and Wilson et al. (2013) [12]:

$$\text{DXA} - \text{BV}_{\text{Silva}}\ (\text{L}) = \left(\frac{FM}{0.89}\right) + \left(\frac{LST}{1.05}\right) + \left(\frac{BMC}{7.81}\right) - 0.126 \quad (1)$$

$$\text{DXA} - \text{BV}_{\text{Wilson}}\ (\text{L}) = \left(\frac{FM}{0.88}\right) + \left(\frac{LST}{1.05}\right) + \left(\frac{BMC}{4.85}\right) + 0.01 \quad (2)$$

It is important to mention that even though Hologic DXA models were used to create these two equations, there were differences in the analytical methods employed. Wilson et al. (2013) utilized the NHANES correction factor [25], so when processing the data, the variables in this equation underwent adjustment beforehand with this correction factor (corrected LST was obtained by multiplying the DXA-derived LST by 0.946, and corrected FM was calculated by subtracting the sum of corrected LST and BMC from total DXA mass) [25].

DXA BV was also estimated following a theory-based approach [9], using the value for the density of the lean soft tissue (LST) proposed by Heymsfield and colleagues [9] (1.064 kg/L) and confirmed by our laboratory [18] in a sample of 201 athletes (36.3% females).

$$DXA - BV_{Heymsfield}\ (L) = \left(\frac{FM}{0.9007}\right) + \left(\frac{LST}{1.064}\right) + \left(\frac{Mo}{2.982}\right) \quad (3)$$

2.2.4 | Air Displacement Plethysmography (ADP)

BV was determined using an ADP (BOD POD model 2007A version 5.4.3, software BOD POD Body Composition System, COSMED, Albano Laziale, Italy). Each participant wore a bathing suit, and their BW was measured using an electronic scale connected to the plethysmograph computer. BV was computed by adjusting the initial BV for thoracic gas volume, measured for all participants. The CV value in our laboratory for measuring BV is 0.4% [5].

Subsequently, body density was calculated as the ratio of BW to BV. FM was estimated from body density based on a 2C model proposed by Siri [28] ($2C_{\text{Siri}}$) and Brozek [4] ($2C_{\text{Brozek}}$):

$$2C_{Siri}\ (kg) = 4.95 * BV - 4.50 * BM \quad (4)$$

$$2C_{Brozek}\ (kg) = 4.570 * BV - 4.142 * BM \quad (5)$$

The Siri equation assumes that the densities of FM and FFM are 0.900 g/cm^3 and 1.100 g/cm^3, respectively. The primary difference between Siri (1961) and Brozek (1963) equations is the assumed temperature and corresponding density of FM: the Siri equation assumes an FM density of 0.900 g/cm^3 at 37°C, whereas the Brozek equation uses an FM density of 0.9007 g/cm^3 at 36°C [4, 28].

2.2.5 | Total-Body Water (TBW)

TBW was measured using the criterion method of deuterium dilution, analyzed with a stable gas isotope ratio mass spectrometer (Hydra 20–20, PDZ, software SerCon Control Callisto v40.0.11, Europa Scientific, UK). After a 12-h fast, an initial urine sample was collected, followed by the administration of a dose of deuterium oxide solution corresponding to 0.1 g/kg of BW. A second urine sample was collected after a 4-h equilibration period. The CV in our laboratory for TBW measurement, determined through a test–retest analysis involving ten subjects, stands at 0.3% [5].

2.2.6 | Bioelectrical Impedance Analysis (BIA)

Bioelectrical impedance spectroscopy (BIS; model 4000B; Xitron Technologies, San Diego, CA) was used as an alternative to deuterium dilution to determine TBW. BIS-derived TBW was calculated using the inherent BIS calculation based on Cole modeling [29] and mixture theories [30] rather than regression equations. A test–retest analysis of ten healthy participants showed a CV of 0.3% for BIS-derived TBW.

Additionally, using the aforementioned device, whole-body resistance (R) at 50 kHz was measured to accommodate laboratories without a BIS device. This resistance data was used to estimate TBW using Matias et al. [31] and Sun et al. [32] equations. In our laboratory, the CV for R is 0.6%.

2.2.7 | Criterion Four-Compartment Body Composition Model

A criterion 4-compartment model was used to assess FM based on the equation described by Wang et al. (2002) [33]:

$$\text{FM (kg)} = 2.748 \times \text{BV} - 0.699 \times \text{TBW} + 1.129 \times \text{Mo} - 2.051 \times \text{BW} \quad (6)$$

Where BV is body volume (L) measured by ADP, TBW is total body water (kg) by deuterium dilution, Mo is bone mineral (kg) by DXA, and BW is body weight (kg).

2.2.8 | Rapid Four-Compartment Body Composition Models

A modified 4C model was used to determine FM by incorporating a DXA-derived BV value as previously described (Equations 1–3). Additionally, a simplified and faster model version was developed, using BV derived from DXA instead of ADP, and TBW derived from BIS and models based on single-frequency BIA devices instead of deuterium dilution. Table 1 summarizes the several rapid 4C models evaluated.

2.2.9 | Propagation of Measurement Error

In this study, the error associated with measuring FM from the criterion 4C model can be estimated by assuming an

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TABLE 1 | Variations tested in rapid four-compartment body composition models.

Model version	TBW	BV	Mo	BW
Criterion 4C model	Deuterium	ADP	DXA	Scale
4C TBW_{BIS}	BIS	ADP	DXA	Scale
4C TBW_{Matias}	Matias et al.	ADP	DXA	Scale
4C TBW_{Sun}	Sun et al.	ADP	DXA	Scale
4C $BV_{Silva}TBW_{BIS}$	BIS	DXA-BV_{Silva}	DXA	Scale
4C $BV_{Wilson}TBW_{BIS}$	BIS	DXA-BV_{Wilson}	DXA	Scale
4C $BV_{Heymsfield}TBW_{BIS}$	BIS	DXA-$BV_{Heymsfield}$	DXA	Scale
4C $BV_{Silva}TBW_{Matias}$	Matias et al.	DXA-BV_{Silva}	DXA	Scale
4C $BV_{Wilson}TBW_{Matias}$	Matias et al.	DXA-BV_{Wilson}	DXA	Scale
4C $BV_{Heymsfield}TBW_{Matias}$	Matias et al.	DXA-$BV_{Heymsfield}$	DXA	Scale
4C $BV_{Silva}TBW_{Sun}$	Sun et al.	DXA-BV_{Silva}	DXA	Scale
4C $BV_{Wilson}TBW_{Sun}$	Sun et al.	DXA-BV_{Wilson}	DXA	Scale
4C $BV_{Heymsfield}TBW_{Sun}$	Sun et al.	DXA-$BV_{Heymsfield}$	DXA	Scale

Note: Matias et al.: TBW = $0.286 \times 0.195 \times S^2/R + 0.385 \times Wt + 5.086 \times Sex$. Sun et al.: Male, TBW = $1.20 + 0.45 \times S^2/R + 0.18 \times Wt$; Female, TBW = $3.75 + 0.45 \times S^2/R + 0.11 \times Wt$. Where sex is 0 if female or 1 if male, Wt is weight in kg, S is stature in cm, R is resistance in ohm (Ω).
Abbreviations: 4C, four-compartment; BIS, bioelectrical impedance spectroscopy; BV, body volume; BW, body weight; DXA, dual-energy x-ray absorptiometry; Mo, mineral content; SF-BIA, single-frequency bioelectrical impedance analysis; TBW, total body water.

average body composition of the whole sample (BV, TBW, Mo, and weight) and measurement precision of each method, as stated throughout this section.

$$\begin{aligned} FM\ \upsilon 2 \ = \ & (2.748 \text{ x } 69.0 \text{ x } 0.004)^2 + (0.699 \text{ x } 44.54 \text{ x } 0.003)^2 \\ & + (1.129 \text{ x } 2.88 \text{ x } 0.013)^2 + (2.051 \text{ x } 73.15 \text{x } 0.0007)^2 \\ & = 0.60 \ (\upsilon \simeq 0.77 \ kg). \end{aligned}$$

The precision of the 4C model to determine FM was ≃0.77 kg.

2.3 | Statistical Analysis

IBM SPSS Statistics (IBM, Chicago, Illinois, USA) version 29.0 for Windows was used for data analysis. Statistical significance was set at $p < 0.05$ (two-sided). The data distribution and normality was evaluated by analyzing skewness and kurtosis, with all variables falling within acceptable ranges of |skewness| < 3 and |kurtosis| < 8 [34]. Descriptive statistics were performed and presented as mean (standard deviation [SD]).

Paired samples *t*-tests were performed to evaluate the differences between the criterion 4C and the rapid 4C model estimations for FM (kg). The effect size (ES) of the differences was determined using Cohen's *d* for paired samples [35] and classified according to Hopkin's scale [36]: [0; 0.2 [= trivial, [0.2; 0.6 [= small, [0.6; 1.2 [= moderate, [1.2; 2.0 [= large, ≥2.0 = very large. Mean differences (MD) were calculated by subtracting the value from the alternative estimation from the value obtained using the criterion 4C model. Positive differences indicate underestimation, while negative differences indicate overestimation. Equivalence testing was performed to assess the agreement between criterion 4C and the rapid 4C model estimations for FM (kg). The mean difference between the paired observations for each rapid 4C model estimate, along with its 90% confidence interval (CI) of the difference, was calculated. This interval was compared to an equivalence region of 5% of the mean criterion 4C FM. The rapid 4C model estimates are considered significantly equivalent to the criterion with $p < 0.05$ when the 90% CI of the difference is completely within the designated equivalence region [37]. For FM% comparisons, the standard error of the estimate (SEE) was also calculated and interpreted according to standards proposed by Heyward et al. [38].

Furthermore, the agreement between criterion 4C and the alternative estimations was evaluated using the Bland–Altman method [39], including the calculation of the 95% limits of agreement (LOA). This analysis also included the analysis of the correlation between the mean values and the differences to detect potential proportional bias. These calculations were performed using GraphPad Prism software version 10.4.1.

3 | Results

Table 2 presents the participants' characteristics and body composition. Male participants were taller, heavier, and had greater FFM ($p < 0.001$). In contrast, female participants had higher FM and %FM ($p < 0.001$).

3.1 | DXA-Derived BV Equations

Regarding the whole sample ($n = 143$), DXA-BV_{Silva} did not differ significantly ($p = 0.290$; ES [95% CI] = −0.09 [−0.25; 0.08]; MD = −0.04 L, SD = 0.48) compared to the criterion. In contrast,

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DXA-BV_{Wilson} and DXA-$BV_{Heymsfield}$ estimated significantly different BV ($p < 0.001$; MD = −1.13 L, SD = 0.50, and $p = 0.028$; MD = 0.09 L, SD = 0.49) with very large and trivial effect sizes (ES [95% CI] = −2.2 [−2.5; −1.9] and ES [95% CI] = 0.18 [0.02; 0.35]), respectively.

The comparison between DXA-derived BV estimates and ADP-BV, for females and males, is presented in Table 3. Our equation (DXA-BV_{Silva}) and DXA-$BV_{Heymsfield}$ provided reliable BV estimates (Table 3), with very similar mean differences and narrow 95% limits of agreement in both females and males. In contrast, the DXA-BV_{Wilson} equation presented significant mean differences for both sexes (females: $p < 0.001$; MD = −1.08 L, SD = 0.38; males: $p < 0.001$; MD = −1.15 L, SD = 0.55), with very large ES (females: ES [95% CI] = −2.86 [−3.57;-2.14]; males: ES [95% CI] = −2.10 [−2.45; −1.76]).

TABLE 2 | Participants' characteristics and body composition.

	Whole sample ($n = 143$)	Female ($n = 39$)	Male ($n = 104$)
Age (years)	21.1 (4.8)	19.6 (5.4)	21.6 (4.5)
Weight (kg)	73.2 (10.7)	65.7 (8.3)	76.0 (10.1)
Height (cm)	179.4 (9.8)	172.8 (7.9)	181.9 (9.4)
BMI (kg/m^2)	22.7 (2.2)	22.0 (2.2)	22.9 (2.2)
BMC (kg)	2.8 (0.6)	2.4 (0.4)	3.0 (0.6)
4C Fat mass (kg)	10.8 (5.4)	15.8 (5.2)	8.9 (4.1)
4C Fat mass (%)	14.8 (7.2)	23.6 (5.7)	11.5 (4.4)
4C FFM (kg)	62.4 (10.7)	49.9 (5.3)	67.1 (8.2)

Note: Data is presented as mean (SD).
Abbreviations: 4C, four-compartment; BMC, bone mineral content; BMI, body mass index; FFM, fat-free mass; LST, lean soft tissue; SD, standard deviation.

TABLE 3 | Validation of DXA-derived BV estimates for females and males.

						Bland–Altman		
					ES	95% LOA		
Method	Mean (SD)	MD (SD)	p	r (p)	Cohen' d [95% confidence interval]	Lower	Upper	r (p)
Females ($n = 39$)								
ADP-BV	63.01 (8.39)							
DXA-BV_{Silva}	63.10 (8.38)	−0.08 (0.35)	0.145	0.999 (< 0.001)	−0.24 [−0.56; 0.08]	−0.78	0.61	0.033 (0.844)
DXA-BV_{Wilson}	64.09 (8.49)	−1.08 (0.38)	< 0.001	0.999 (< 0.001)	−2.86 [−3.57;-2.14]	−1.82	−0.34	−0.266 (0.105)
DXA-$BV_{Heymsfield}$	62.95 (8.33)	0.06 (0.39)	0.306	0.999 (< 0.001)	0.17 [−0.15; 0.48]	−0.69	0.82	0.153 (0.353)
Males ($n = 104$)								
ADP-BV	71.07 (9.69)							
DXA-BV_{Silva}	71.10 (9.70)	−0.03 (0.52)	0.600	0.99 (< 0.001)	0.05 [−0.24; 0.14]	−1.04	0.99	−0.023 (0.821)
DXA-BV_{Wilson}	72.22 (9.84)	−1.15 (0.55)	< 0.001	0.999 (< 0.001)	−2.10 [−2.45;-1.76]	−2.22	−0.08	−0.278 (0.004)
DXA-$BV_{Heymsfield}$	70.97 (9.68)	0.10 (0.53)	0.052	0.999 (< 0.001)	0.19 [0.00; 0.39]	−0.94	1.14	0.014 (0.888)

Note: Comparison of means between methods was performed using the paired sample t-test. Pearson's correlation was performed to assess the correlation between methods and between the mean and the difference of the methods (Bland–Altman graph).
Abbreviations: 95% LOA, 95% limits of agreement (MD ± 1.96 × SD of differences); ADP-BV, air displacement plethysmograph body volume; BMC, bone mineral content; BV, body volume; ES, effect size; MD, mean difference (subtraction of the value obtained by DXA-derived equations from the value estimated by ADP-BV); r, Pearson's correlation coefficient; SD, standard deviation.

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3.2 | Rapid 4C Model Estimates

The validity of the alternative FM estimates (kg) when compared to the criterion 4C model is presented in Table 4 (whole sample [$n = 115$]). The mean FM measured by the criterion 4C model was 11.77 (SD = 5.33) kg. The mean values of the 15 alternative estimates ranged from 11.25 (SD = 4.68) kg for 4C $BV_{Heymsfield}TBW_{Matias}$ to 15.45 (SD = 5.31) kg for 4C $BV_{Wilson}TBW_{Sun}$. Strong and very strong correlations were observed for all FM estimations, ranging from 0.893 ($p < 0.001$) [DXA] to 0.975 ($p < 0.001$) [4C TBW_{BIS}].

The Bland–Altman analysis revealed that 4C TBW_{BIS} displayed the narrowest 95% LOA (−2.62 to 2.27 kg) for individual FM differences. Among the rapid 4C models using BV estimations from DXA, 4C $BV_{Silva}TBW_{BIS}$ displayed the narrowest 95% LOA (−3.79 to 3.33 kg) with no proportional bias. These rapid 4C models also agreed better with the criterion than 2C ($2C_{Siri}$ and $2C_{Brozek}$) and 3C models (DXA).

The comparison of FM estimates (kg) for females and males can be consulted in Tables 5 and 6, respectively.

Regarding females, the 4C TBW_{BIS} displayed the narrowest 95% LOA (−3.16 to 1.65 kg), with the 4C $BV_{Silva}TBW_{BIS}$ also demonstrating narrow 95% LOA (−4.32 to 1.7 kg) with no proportional bias. In contrast, the 4C $BV_{Heymsfield}TBW_{Matias}$ method, despite showing very small mean differences (MD = −0.03 kg; SD = 2.41 kg), had significantly wider 95% LOA (−4.75 to 4.70 kg) and exhibited a moderate proportional bias ($r = 0.663$, $p < 0.001$).

For males, 4C TBW_{BIS} also displayed the narrowest 95% LOA (−2.14 to 2.38 kg), with no proportional bias. Among the rapid 4C models using both BV and TBW alternative estimates, 4C $BV_{Silva}TBW_{BIS}$ displayed the narrowest 95% LOA (−3.28 to 3.61 kg), with no proportional bias.

The comparison of FM estimates (%) for the whole sample can be consulted in Table 7. The 4C TBW_{BIS} provided the lowest SEE (1.66%), categorized as ideal. In contrast, 4C $BV_{Heymsfield}TBW_{Matias}$ and DXA provided the highest SEE (3.32% for both), categorized as very good to good.

The comparison of FM estimates (%) for females and males can be consulted in Tables S1 and S2, respectively.

4 | Discussion

Our study demonstrates that the rapid 4C model estimates FM more accurately than 2C ($2C_{Siri}$ and $2C_{Brozek}$) and 3C (DXA) models when compared to the criterion multicompartment approach. The rapid 4C models presented are faster, more accessible, and avoid needing more expensive and uncommon equipment. This practical alternative utilizes two increasingly common devices in sports settings [40]—DXA and BIA—facilitating FM assessment without requiring athletes to move between measurements.

The DXA-BV_{Silva} and DXA-$BV_{Heymsfield}$ equations provided reliable BV estimates both at a group and individual level, with 95% limits of agreement narrower than those reported by other authors [10, 13]. These findings enhance the robustness of the DXA-BV_{Silva} equation, validated here in a diverse and independent sample of athletes. Minor group and individual differences between DXA-derived BV and ADP suggest that BV can be accurately estimated by using a single DXA scan.

The literature on rapid 4C models for athletes is limited and most rapid 4C studies to date rely on BIS for TBW estimation rather than gold-standard dilution techniques [10, 13, 15, 16, 41]. To address this, deuterium dilution was employed to measure TBW, allowing a thorough evaluation of alternative measures for both BV and TBW.

Fifteen alternative methods for estimating FM were evaluated in a cohort of athletes from various sports, finding widely varying results. Among these, the rapid 4C model using ADP-derived BV and TBW derived from BIS (4C TBW_{BIS}) demonstrated the most accurate estimate of FM, with the narrowest 95% limits of agreement. However, it is crucial to clarify that, while 4C TBW_{BIS} provided the closest approximation, using BIS to assess TBW may potentially introduce another source of error. For instance, using this rapid alternative, the actual FM value of a male athlete with 10 kg could range from 7.9 kg to 12.4 kg.

Among the rapid 4C models using BV estimations from DXA, the 4C $BV_{Silva}TBW_{BIS}$ displayed the narrowest 95% LOA. These results also compare favorably to findings from studies on individuals with overweight or obesity [15], adults with varying BMI and waist circumferences [16], and adults whose BMI ranged from normal to obese [13]. Additionally, the mean differences were relatively small and nonsignificant (MD = −0.23 kg; SD = 1.82 kg; ES [95% CI] = −0.13 [−0.31; 0.06]). Equivalence testing at a 5% interval showed that 4C $BV_{Silva}TBW_{BIS}$ and 4C $BV_{Heymsfield}TBW_{BIS}$ were equivalent for the whole sample but not when split by sex. While the literature lacks consensus on the optimal interval (5% or 10%), we used a stricter 5% threshold for stronger support. Notably, a 10% interval would have shown equivalence for both models across the whole sample and in both sexes.

Our findings also indicate sex discrepancies in the accuracy of the rapid 4C models. In females, these methods significantly overestimated mean FM, while in males a slight, nonsignificant underestimation was observed for most models. The 95% limits of agreement were also broader in females. Recognizing that FM estimation from these alternative models relies on alternative TBW and BV estimates, the differences between the error (reference minus alternative estimation) of TBW and BV estimation between sexes were analyzed. While BV error did not differ significantly between males and females, TBW estimation did ($p < 0.001$; ES [95% CI] = 0.75 [0.35; 1.14]). Indeed, BIS underestimated TBW in females ($p < 0.001$; MD = 1.1 kg; SD = 1.8 kg; ES [95% CI] = 0.62 [0.27; 0.96]), mirroring the findings of Quitério et al. (2009) [42] in adolescent athletes, who observed that BIS-derived TBW differed significantly from deuterium dilution in girls, whereas in boys no differences were found. Using a 4C model, if TBW in females is underestimated—assuming BW is accurately measured—the compensatory effect would result in an overestimation of FM in this group.

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TABLE 4 | Comparison of FM (kg) values between the criterion 4C model and alternative estimates.

				ES	Bland–Altman			Equivalence testing[a]	
					95% LOA				
4C model version ($n = 115$)	**Mean (SD)**	**MD (SD)**	***p***	**Cohen' *d* [95% confidence interval]**	**LL**	**UL**	***r* (*p*)**	**Eq.**	**90% CI difference**
Criterion 4C model	11.77 (5.33)								
4C TBW_{BIS}	11.95 (5.58)	−0.18 (1.25)	0.131	−0.14 [−0.33; 0.04]	−2.62	2.27	−0.199 (0.033)	Yes	[−0.37; 0.02]
4C TBW_{Matias}	11.60 (4.71)	0.17 (1.75)	0.306	0.10 [−0.09; 0.28]	−3.26	3.61	0.356 (<0.001)	Yes	[−0.10; 0.44]
4C TBW_{Sun}	12.42 (5.16)	−0.65 (1.48)	<0.001	−0.44 [−0.63; −0.25]	−3.56	2.26	0.112 (0.232)	No	[−0.88; −0.42]
4C $BV_{Silva}TBW_{BIS}$	12.00 (5.54)	−0.23 (1.82)	0.182	−0.13 [−0.31; 0.06]	−3.79	3.33	−0.120 (0.201)	Yes	[−0.51; 0.05]
4C $BV_{Wilson}TBW_{BIS}$	14.97 (5.66)	−3.20 (1.83)	<0.001	−1.75 [−2.04; −1.46]	−6.78	0.37	−0.183 (0.050)	No	[−3.49; −2.92]
4C $BV_{Heymsfield}TBW_{BIS}$	11.59 (5.49)	0.18 (1.87)	0.308	0.10 [−0.09; 0.28]	−3.48	3.83	−0.086 (0.363)	Yes	[−0.11; 0.47]
4C $BV_{Silva}TBW_{Matias}$	11.65 (4.74)	0.12 (2.34)	0.592	0.05 [−0.13; 0.23]	−4.48	4.71	0.258 (0.005)	Yes	[−0.24; 0.48]
4C $BV_{Wilson}TBW_{Matias}$	14.63 (4.86)	−2.86 (2.32)	<0.001	−1.23 [−1.48; −0.99]	−7.40	1.68	0.209 (0.025)	No	[−3.22; −2.50]
4C $BV_{Heymsfield}TBW_{Matias}$	11.25 (4.68)	0.52 (2.41)	0.022	0.22 [0.03; 0.40]	−4.19	5.24	0.276 (0.003)	No	[0.15; 0.90]
4C $BV_{Silva}TBW_{Sun}$	12.47 (5.19)	−0.70 (2.16)	<0.001	−0.33 [−0.51; −0.14]	−4.93	3.52	0.066 (0.483)	No	[−1.04; −0.37]
4C $BV_{Wilson}TBW_{Sun}$	15.45 (5.31)	−3.68 (2.16)	<0.001	−1.70 [−1.99; −1.41]	−7.92	0.56	−0.008 (0.933)	No	[−4.02; −3.35]
4C $BV_{Heymsfield}TBW_{Sun}$	12.07 (5.13)	−0.30 (2.20)	0.149	−0.14 [−0.32; 0.05]	−4.61	4.02	0.093 (0.322)	No	[−0.64; 0.04]
DXA total FM	13.18 (4.89)	−1.42 (2.40)	<0.001	−0.59 [−0.79; −0.39]	−6.12	3.29	0.186 (0.047)	No	[−1.95; −1.28]
$2C_{Siri}$	12.50 (5.04)	−0.73 (2.07)	<0.001	−0.35 [−0.54; −0.16]	−4.78	3.32	0.141 (0.131)	No	[−1.05; −0.41]
$2C_{Brozek}$	12.53 (4.69)	−0.76 (2.07)	<0.001	−0.37 [−0.56; −0.18]	−4.82	3.3	0.313 (0.007)	No	[−1.08; −0.44]

Note: A comparison of means between methods was performed using the paired sample *t*-test. Pearson's correlation (*r*) was performed to assess the correlation between methods and between the mean and the difference of the methods (Bland–Altman graph).

Abbreviations: 95% LOA, 95% limits of agreement (MD ± 1.96 × SD of differences); BV, body volume; CI, confidence interval; DXA, dual-energy x-ray absorptiometry; Eq., equivalence to criterion 4C model; ES, effect size; LL, lower limit; MD, mean difference (subtraction of the value obtained by the alternative estimates from the value estimated by criterion); SD, standard deviation; TBW, total body water; UL, upper limit.

[a]Based on 5% equivalence interval, which corresponds to 0.59 kg.

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TABLE 5 | Comparison of FM (kg) values between the criterion 4C model and alternative estimates in females.

				ES	Bland–Altman			Equivalence testing[a]	
					95% LOA				
4C model version ($n = 39$)	**Mean (SD)**	**MD (SD)**	***p***	**Cohen' *d* [95% confidence interval]**	**LL**	**UL**	***r* (*p*)**	**Eq.**	**90% CI difference**
Criterion 4C model	15.80 (5.21)								
4C TBW_{BIS}	16.56 (5.12)	−0.76 (1.23)	<0.001	−0.62 [−0.96; −0.27]	−3.16	1.65	0.072 (0.663)	No	[−1.09; −0.43]
4C TBW_{Matias}	16.00 (3.83)	−0.20 (1.82)	0.487	−0.11 [−0.43; 0.20]	−3.76	3.35	0.764 (<0.001)	Yes	[−0.70; 0.29]
4C TBW_{Sun}	16.72 (4.83)	−0.92 (1.43)	<0.001	−0.65 [−0.99; −0.30]	−3.72	1.87	0.260 (0.260)	No	[−1.31; −0.54]
4C $BV_{Silva}TBW_{BIS}$	16.79 (5.05)	−0.99 (1.70)	<0.001	−0.59 [−0.92; −0.24]	−4.32	1.70	0.096 (0.561)	No	[−1.45; −0.53]
4C $BV_{Wilson}TBW_{BIS}$	19.53 (5.29)	−3.73 (1.74)	<0.001	−2.15 [−2.72; −1.57]	−7.14	0.33	−0.050 (0.763)	No	[−4.20; −3.26]
4C $BV_{Heymsfield}TBW_{BIS}$	16.38 (4.91)	−0.58 (1.80)	0.050	−0.33 [−0.65; 0.00]	−4.11	2.94	0.169 (0.303)	No	[−1.07; −0.10]
4C $BV_{Silva}TBW_{Matias}$	16.23 (3.79)	−0.44 (2.27)	0.238	−0.19 [−0.51; 0.13]	−4.89	4.02	0.636 (<0.001)	No	[−1.05; 0.18]
4C $BV_{Wilson}TBW_{Matias}$	18.97 (4.06)	−3.17 (2.21)	<0.001	−1.44 [−1.88; −0.98]	−7.50	1.15	0.531 (<0.001)	No	[−3.77; −2.58]
4C $BV_{Heymsfield}TBW_{Matias}$	15.83 (3.64)	−0.03 (2.41)	0.942	−0.01 [−0.33; 0.30]	−4.75	4.70	0.663 (<0.001)	Yes	[−0.68; 0.62]
4C $BV_{Silva}TBW_{Sun}$	16.95 (4.81)	−1.15 (1.99)	<0.001	−0.58 [−0.92; −0.24]	−5.05	2.75	0.204 (0.214)	No	[−1.69; −0.62]
4C $BV_{Wilson}TBW_{Sun}$	19.69 (5.07)	−3.89 (2.04)	<0.001	−1.91 [−2.43; −1.37]	−7.89	0.11	0.067 (0.687)	No	[−4.44; −3.34]
4C $BV_{Heymsfield}TBW_{Sun}$	16.54 (4.66)	−0.75 (2.08)	0.031	−0.36 [−0.68; −0.03]	−4.82	3.33	0.266 (0.101)	No	[−1.31; −0.18]
DXA total FM	17.07 (4.43)	−1.27 (1.93)	<0.001	−0.66 [−1.00; −0.31]	−5.05	2.52	0.411 (0.009)	No	[−1.79; −0.75]
$2C_{Siri}$	16.13 (4.99)	−0.33 (1.61)	0.209	−0.21 [−0.52; 0.11]	−3.48	2.82	0.139 (0.398)	Yes	[−0.76; 0.11]
$2C_{Brozek}$	15.78 (4.69)	0.02 (1.61)	0.942	0.01 [−0.30; 0.33]	−3.14	3.18	0.324 (0.045)	Yes	[−0.42; 0.45]

Note: A comparison of means between methods was performed using the paired sample *t*-test. Pearson's correlation (*r*) was performed to assess the correlation between methods and between the mean and the difference of the methods (Bland–Altman graph).

Abbreviations: 95% LOA, 95% limits of agreement (MD ± 1.96 x SD of differences); BV, body volume; CI, confidence interval; DXA, dual-energy x-ray absorptiometry; Eq., equivalence to criterion 4C model; ES, effect size; LL, lower limit; MD, mean difference (subtraction of the value obtained by the alternative estimates from the value estimated by criterion); SD, standard deviation; TBW, total body water; UL, upper limit.

[a]Based on 5% equivalence interval, which corresponds to 0.79 kg.

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TABLE 6 | Comparison of FM (kg) values between the criterion 4C model and alternative estimates in males.

				ES	Bland–Altman			Equivalence testing[a]	
					95% LOA				
4C model version ($n = 76$)	**Mean (SD)**	**MD (SD)**	***p***	**Cohen' *d* [95% confidence interval]**	**LL**	**UL**	***r* (*p*)**	**Eq.**	**90% CI difference**
Criterion 4C model	9.70 (4.08)								
4C TBW_{BIS}	9.58 (4.16)	0.12 (1.15)	0.358	0.11 [−0.12; 0.33]	−2.14	2.38	−0.065 (0.579)	Yes	[−0.10; 0.34]
4C TBW_{Matias}	9.34 (3.32)	0.36 (1.70)	0.069	0.21 [−0.02; 0.44]	−2.97	3.70	0.453 (<0.001)	No	[0.03; 0.68]
4C TBW_{Sun}	10.22 (3.75)	−0.52 (1.50)	0.004	−0.34 [−0.57; −0.11]	−3.47	2.44	0.218 (0.059)	No	[−0.80; −0.23]
4C $BV_{Silva}TBW_{BIS}$	9.54 (3.97)	0.17 (1.76)	0.416	0.09 [−0.13; 0.32]	−3.28	3.61	0.067 (0.568)	No	[−0.17; 0.50]
4C $BV_{Wilson}TBW_{BIS}$	12.64 (4.26)	−2.94 (1.82)	<0.001	−1.61 [−1.95; −1.27]	−6.51	0.64	−0.101 (0.384)	No	[−3.28; −2.59]
4C $BV_{Heymsfield}TBW_{BIS}$	9.13 (3.94)	0.57 (1.79)	0.007	0.32 [0.09; 0.55]	−2.93	4.07	0.083 (0.083)	No	[0.23; 0.91]
4C $BV_{Silva}TBW_{Matias}$	9.30 (3.22)	0.40 (2.34)	0.139	0.17 [−0.06; 0.40]	−4.19	5.00	0.386 (0.386)	No	[−0.05; 0.85]
4C $BV_{Wilson}TBW_{Matias}$	12.40 (3.56)	−2.70 (2.37)	<0.001	−1.14 [−1.43; −0.85]	−7.34	1.95	0.232 (0.044)	No	[−3.15; −2.25]
4C $BV_{Heymsfield}TBW_{Matias}$	8.90 (3.18)	0.81 (2.37)	0.004	0.34 [0.11; 0.57]	−3.84	5.45	0.398 (0.000)	No	[0.35; 1.26]
4C $BV_{Silva}TBW_{Sun}$	10.17 (3.66)	−0.47 (2.21)	0.067	−0.21 [−0.44; 0.02]	−4.81	3.87	0.196 (0.090)	No	[−0.90; −0.05]
4C $BV_{Wilson}TBW_{Sun}$	13.27 (3.96)	−3.57 (2.23)	<0.001	−1.60 [−1.94; −1.26]	−7.95	0.80	0.055 (0.635)	No	[−4.00; −3.15]
4C $BV_{Heymsfield}TBW_{Sun}$	9.77 (3.63)	−0.07 (2.24)	0.790	−0.03 [−0.26; 0.19]	−4.46	4.32	0.209 (0.070)	No	[−0.50; 0.36]
DXA total FM	11.19 (3.82)	−1.49 (2.62)	<0.001	−0.57 [−0.81; −0.33]	−6.62	3.65	0.103 (0.375)	No	[−1.99; −0.99]
$2C_{Siri}$	10.64 (3.96)	−0.93 (2.25)	<0.001	−0.42 [−0.65; −0.18]	−5.34	3.47	0.054 (0.640)	No	[−1.36; −0.51]
$2C_{Brozek}$	10.86 (3.75)	1.16 (2.17)	<0.001	−0.54 [−0.77; −0.29]	−5.42	3.10	0.160 (0.168)	No	[−1.58; −0.75]

Note: A comparison of means between methods was performed using the paired sample *t*-test. Pearson's correlation (*r*) was performed to assess the correlation between methods and between the mean and the difference of the methods (Bland–Altman graph).

Abbreviations: 95% LOA, 95% limits of agreement (MD ± 1.96 × SD of differences); BV, body volume; CI, confidence interval; DXA, dual-energy x-ray absorptiometry; Eq., equivalence to criterion 4C model; ES, effect size; LL, lower limit; MD, mean difference (subtraction of the value obtained by the alternative estimates from the value estimated by criterion); SD, standard deviation; TBW, total body water; UL, upper limit.

[a]Based on 5% equivalence interval, which corresponds to 0.49 kg.

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TABLE 7 | Comparison of FM (%) values between the criterion 4C model and alternative estimates.

				ES		Bland–Altman			Equivalence testing[a]	
						95% LOA				
4C model version ($n = 115$)	**Mean (SD)**	**MD (SD)**	***p***	**Cohen' *d* [95% confidence interval]**	**SEE**	**LL**	**UL**	***r* (*p*)**	**Eq.**	**90% CI difference**
Criterion 4C model	16.2 (7.1)									
4C TBW_{BIS}	16.5 (7.6)	−0.3 (1.7)	0.072	−0.17 [−0.35; 0.02]	1.56	−3.6	3.0	−0.297 (0.001)	Yes	[−0.5; 0.0]
4C TBW_{Matias}	16.1 (6.6)	0.2 (2.5)	0.493	0.06 [−0.12; 0.25]	2.46	−4.7	5.0	0.232 (0.012)	Yes	[−0.2; 0.5]
4C TBW_{Sun}	17.2 (7.0)	−0.9 (2.1)	< 0.001	−0.46 [−0.65; −0.27]	2.06	−5.0	3.1	0.071 (0.452)	No	[−1.3; −0.6]
4C $BV_{Silva}TBW_{BIS}$	16.6 (7.7)	−0.4 (2.5)	0.115	−0.15 [−0.33; 0.04]	2.32	−5.3	4.5	−0.226 (0.015)	Yes	[−0.8; 0.0]
4C $BV_{Wilson}TBW_{BIS}$	20.7 (7.7)	−4.4 (2.5)	< 0.001	−1.75 [−2.04; −1.46]	2.35	−9.4	2.5	−0.240 (0.010)	No	[−4.8; −4.1]
4C $BV_{Heymsfield}TBW_{BIS}$	16.0 (7.6)	0.2 (2.6)	0.432	0.07 [−0.11; 0.26]	2.40	−4.8	5.2	−0.194 (0.038)	Yes	[−0.2; 0.6]
4C $BV_{Silva}TBW_{Matias}$	16.1 (6.7)	0.1 (3.2)	0.806	0.02 [−0.16; 0.21]	3.24	−6.3	6.4	0.130 (0.165)	Yes	[−0.4; 0.6]
4C $BV_{Wilson}TBW_{Matias}$	20.2 (6.8)	−4.0 (3.3)	< 0.001	−1.22 [−1.46; −0.98]	3.27	−10.4	2.4	0.112 (0.234)	No	[−4.5; −3.5]
4C $BV_{Heymsfield}TBW_{Matias}$	15.6 (6.7)	0.6 (3.3)	0.044	0.19 [0.01; 0.37]	3.32	−5.9	7.1	0.147 (0.118)	No	[0.1; 1.1]
4C $BV_{Silva}TBW_{Sun}$	17.2 (7.1)	−1.0 (3.0)	< 0.001	−0.35 [−0.53; −0.16]	2.92	−6.8	4.8	−0.002 (0.983)	No	[−1.5; −0.6]
4C $BV_{Wilson}TBW_{Sun}$	21.3 (7.2)	−5.1 (3.0)	< 0.001	−1.70 [−1.98; −1.41]	2.94	−11.0	0.8	−0.018 (0.845)	No	[−5.6; −4.6]
4C $BV_{Heymsfield}TBW_{Sun}$	16.7 (7.1)	−0.5 (3.0)	0.101	−0.15 [−0.34; 0.03]	2.99	−6.4	5.5	0.022 (0.818)	No	[−0.9; 0.0]
DXA total FM	18.3 (6.7)	−2.1 (3.3)	< 0.001	−0.63 [−0.83; −0.43]	3.32	−8.6	4.4	0.139 (0.137)	No	[−2.6; −1.6]
$2C_{Siri}$	17.2 (6.8)	−1.0 (2.9)	< 0.001	−0.36 [−0.55; −0.17]	2.87	−6.7	4.6	0.126 (0.182)	No	[−1.5; −0.6]
$2C_{Brozek}$	17.2 (6.3)	−1.0 (2.9)	< 0.001	−0.34 [−0.52; −0.15]	2.87	−6.6	4.7	0.310 (0.001)	No	[−1.4; −0.5]

Note: A comparison of means between methods was performed using the paired sample *t*-test. Pearson's correlation (*r*) was performed to assess the correlation between methods and between the mean and the difference of the methods (Bland–Altman graph).

Abbreviations: 95% LOA, 95% limits of agreement (MD ± 1.96 × SD of differences); BV, body volume; CI, confidence interval; DXA, dual-energy x-ray absorptiometry; Eq., equivalence to criterion 4C model; ES, effect size; LL, lower limit; MD, mean difference (subtraction of the value obtained by the alternative estimates from the value estimated by criterion); SD, standard deviation; SEE, standard error of the estimate; TBW, total body water; UL, upper limit.

[a]Based on 5% equivalence interval, which corresponds to 0.8%.

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The current study uniquely contributes to the existing literature by evaluating alternative measures of TBW in place of the deuterium dilution method. Despite being considered the gold standard, deuterium dilution requires a lengthy process—approximately 4 h per participant—posing significant financial and logistical challenges [43]. Given that athletes often have demanding training schedules and limited free time, methods requiring prolonged assessment periods pose a significant practical challenge. BIS devices, although not reference methods, have been validated against deuterium dilution and provide comparable results much more quickly [44, 45].

Given the occasional unavailability of BIS, this study also tested regression equations derived from raw bioelectrical impedance resistance values (Matias et al. [31] and Sun et al. [32]) to explore alternative TBW measures. Despite generally modest group errors in most comparisons, these regression-derived estimates produced significant individual errors, as indicated by the wide 95% LOA, rendering them less suitable when high accuracy is desired. This underscores the variability and potential limitations of using regression equations for individual TBW estimation in research and clinical applications [45] and highlights the trade-offs between practicality and accuracy in body composition research. Thus, using regression-derived TBW estimates in rapid 4C models is not recommended.

Multi-compartment molecular body composition models are more valid than 2C models because they account for the biological variability in the FFM components [1, 46]. An important consideration is whether the rapid 4C model offers the same advantages as the criterion 4C model over simpler field and laboratory methods. Previous studies in nonathletic samples have yielded mixed results. Some have found that 3C models (DXA) produce similar or smaller errors compared to the rapid 4C model [47], while others have reported greater accuracy with the rapid 4C model [48]. Our new rapid 4C models demonstrated better agreement with the criterion than the 2C ($2C_{Siri}$ and $2C_{Brozek}$) and 3C models (DXA), suggesting that the rapid 4C model retains some of the improved accuracy of the criterion 4C model. Any deviation in the assumed FFM density of 1.1 kg/L introduces errors in body composition estimates derived from 2C models. In our sample ($n = 115$), the mean FFM density was 1.097 kg/L (SD = 0.008), likely accounting for the discrepancies observed in $2C_{Siri}$ and $2C_{Brozek}$ models compared to the criterion method.

The rapid 4C model offers superior accuracy to DXA alone, aligning with findings from Smith-Ryan et al. [48] in healthy, normal-weight young adults. Specifically, in our study, DXA exhibited notably wide 95% LOA (−6.12 to 3.29 kg) and a mean overestimation of approximately 1.5 kg, indicating considerable variability and potential inaccuracies in individual measurements compared to the criterion 4C model. Our findings align with previous studies comparing DXA with criterion multicompartment models in athletes, consistently reporting an overestimation of FM and wide 95% LOA, namely in NCAA Division I collegiate female athletes [49], adolescent athletes [50], and bodybuilders [51]. Additionally, the coefficient of determination ($R^2 = 0.185$) between TBW/FFM and the DXA error (4C—DXA) indicates that variations in FFM hydration account for a notable proportion of these discrepancies. DXA systems fundamentally rely on assumed constant R values for the three compartments [52]. While the R values for fat and bone mineral remain highly stable, primarily due to their simple composition of triglycerides and calcium hydroxyapatite, respectively, the R value for lean soft tissue is potentially more variable. This variability arises from the composition of lean soft tissue, which includes water, protein, and soft tissue minerals, with the stability of its R value depending, at least in part, on the consistency of these constituent proportions [53].

In addition, it is acknowledged that the accuracy of DXA is affected by factors such as the thickness of the tissue and its inability to differentiate soft tissue above or adjacent to bone, potentially leading to underestimated body composition estimates in regions with substantial bone presence, such as the arm and thorax [54, 55].

These limitations likely contribute to the notable bias and significant individual measurement errors observed in athletes. Therefore, caution is warranted when using DXA as the reference method to develop body composition regression equations for field methods, which is common for anthropometric [19] or BIA equations [56].

We acknowledge that, in many sports contexts, DXA may not be readily available to all practitioners, despite the well-established advantages of laboratory-based multicompartment models. Future research should continue to examine field-based 3C models [57, 58], as an accurate measure of TBW—especially in athletic populations—appears to be critically important when assessing body composition.

This study is not without limitations. First, our sample was exclusively composed of athletes aged 16 years and older, so the results may not be applicable to younger athletes. Additionally, female participants represented only a third ($n = 39$) of the sample, underscoring the need for future studies to validate these findings across different age groups and a higher proportion of female athletes. Furthermore, the specific DXA model used in this study may limit the generalizability of the results to other DXA manufacturers and models. Lastly, there is the potential for error in using resistance values from a BIS device in equations originally developed for single-frequency devices, which may impact the accuracy of the TBW estimates. The strengths of this study include its diverse athletic sample across multiple sports, enhancing the representativeness of our findings. Additionally, the external validation of the DXA-derived BV equation in an independent sample enhances the robustness and reliability of our findings. Moreover, our pioneering evaluation of alternative measures of TBW, incorporating both BIS and other published regression models based on raw BIA data, expands the scope of methodological comparison. Finally, this study provides a comprehensive comparison of rapid 4C models against a true criterion 4C model using ADP and deuterium dilution, offering valuable insights into the accuracy and applicability of these models.

In conclusion, our validation of the results of the DXA-BV_{Silva} equation in an independent sample of athletes confirms its robustness and reliability at both group and individual levels. Regarding rapid 4C models, notable differences in fat estimates

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were observed between methods, yet promising alternatives have emerged for both male and female athletes. Among the rapid 4C models using BV estimations from DXA, 4C $BV_{Silva}TBW_{BIS}$ displayed the narrowest 95% limits of agreement. Our research highlights the superior accuracy of rapid multi-compartment models, which account for TBW, compared to 2C ($2C_{Siri}$ and $2C_{Brozek}$) and three-compartment models (DXA), suggesting their potential to replace DXA as the criterion in body composition evaluation where implementing the true criterion 4C model is impractical. This rapid 4C model offers reduced time and equipment requirements, making it particularly attractive for athletes with tight schedules and rigorous training routines.

5 | Perspective

The findings of this study provide strong evidence for using a rapid 4C model in athletes, integrating DXA-derived BV and BIS-derived TBW, as an alternative when implementing the true criterion 4C model is impractical. The study also demonstrates that rapid 4C models provide superior accuracy compared to traditional 2C and 3C methods and are an appealing option for practitioners and athletes. Future research should focus on expanding the application of these models across different athletic populations and evaluating their validity in longitudinal monitoring. Rapid 4C models may bridge the gap between research-grade body composition assessment and real-world sports and clinical settings by reducing equipment and time requirements, ultimately benefiting athletes, coaches, and sports nutrition practitioners.

Author Contributions

T.R.S.: conceptualization, methodology, formal analysis, writing – original draft preparation; R.P.: formal analysis, writing – review and editing; L.B.S.: writing – review and editing, supervision, funding acquisition; V.H.T.: writing – review and editing; A.M.S.: conceptualization, methodology, writing – review and editing, supervision, funding acquisition.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data described in the manuscript will be made available upon reasonable request pending application and approval to analiza@fmh.ulisboa.pt.

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Supporting Information

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SUPPLEMENTARY MATERIAL

Rapid Four-Compartment Models in Athletes Using Alternative Solutions for Body Volume and Water

Tiago R. Silva, Rui Poínhos, Luís B Sardinha, Vitor Hugo Teixeira, Analiza M. Silva

Table S1 Comparison of FM (%) values between the criterion 4C model and alternative estimates in females.

				ES	SEE	Bland-Altman			Equivalence testing [a]	
4C model version (n = 39)	**Mean (SD)**	**MD (SD)**	**p**	**Cohen'd [95% Confidence Interval]**		**95% LOA**		**r (p)**	**Eq.**	**90% CI difference**
						LL	**UL**			
Criterion 4C model	23.6 (5.7)									
4C TBW_{BIS}	24.8 (5.5)	-1.2 (1.8)	<0.001	-0.65 [-0.99; -0.30]	1.85	-4.8	2.4	0.069 (0.676)	No	[-1.7; -0.7]
4C TBW_{Matias}	24.1 (3.4)	-0.5 (2.8)	0.307	-0.17 [-0.48; 0.15]	1.98	-5.9	4.9	0.844 (<0.001)	No	[-1.2; 0.3]
4C TBW_{Sun}	25.1 (4.7)	-1.4 (2.2)	<0.001	-0.65 [-1.00; -0.30]	2.13	-5.7	2.8	0.433 (0.006)	No	[-2.0; -0.8]
4C $BV_{Silva}TBW_{BIS}$	25.2 (5.4)	-1.5 (2.5)	<0.001	-0.62 [-0.96; -0.27]	2.52	-6.5	3.4	0.101 (0.539)	No	[-2.2; -0.9]
4C $BV_{Wilson}TBW_{BIS}$	29.4 (5.4)	-5.7 (2.6)	<0.001	-2.24 [-2.82; -1.64]	2.57	-10.7	-0.7	0.115 (0.488)	No	[-6.4; -5.0]
4C $BV_{Heymsfield}TBW_{BIS}$	24.6 (5.3)	-0.9 (2.7)	0.033	-0.35 [-0.68; -0.03]	2.68	-6.1	4.3	0.151 (0.359)	No	[-1.7; -0.2]
4C $BV_{Silva}TBW_{Matias}$	24.5 (3.3)	-0.8 (3.4)	0.144	-0.24 [-0.56; 0.08]	3.15	-7.5	5.9	0.712 (<0.001)	No	[-1.7; 0.1]
4C $BV_{Wilson}TBW_{Matias}$	28.6 (3.3)	-5.0 (3.5)	<0.001	-1.44 [-1.89; -0.99]	3.21	-11.8	1.8	0.714 (<0.001)	No	[-5.9; -4.1]
4C $BV_{Heymsfield}TBW_{Matias}$	23.9 (3.2)	-0.2 (3.6)	0.721	-0.06 [-0.37; 0.26]	3.32	-7.2	6.8	0.729 (<0.001)	No	[-1.2; 0.8]
4C $BV_{Silva}TBW_{Sun}$	25.4 (4.7)	-1.8 (3.0)	<0.001	-0.59 [-0.93; -0.25]	3.03	-7.6	4.1	0.322 (0.046)	No	[-2.6; -1.0]
4C $BV_{Wilson}TBW_{Sun}$	29.6 (4.7)	-5.9 (3.0)	<0.001	-1.96 [-2.50; -1.42]	3.07	-11.9	0.0	0.333 (0.038)	No	[-6.8; -5.1]
4C $BV_{Heymsfield}TBW_{Sun}$	24.8 (4.6)	-1.2 (3.1)	0.025	-0.37 [-0.70; -0.05]	3.16	-7.3	4.9	0.362 (0.024)	No	[-2.0; -0.3]
DXA total FM	25.8 (4.4)	-2.2 (3.0)	<0.001	-0.73 [-1.08; -0.37]	3.00	-8.0	3.7	0.433 (0.006)	No	[-3.0; -1.4]
$2C_{Siri}$	24.2 (5.4)	-0.5 (2.5)	0.182	-0.22 [-0.53; 0.10]	2.53	-5.5	4.4	0.122 (0.461)	No	[-1.2; 0.1]
$2C_{Brozek}$	23.6 (5.0)	0.1 (2.5)	0.893	0.02 [-0.29; 0.34]	2.53	-4.9	5.0	0.291 (0.072)	Yes	[-0.6; 0.7]

Abbreviations: **BV** body volume. **TBW** total body water. **DXA** dual-energy x-ray absorptiometry. **ES** Effect Size. **MD** mean difference (subtraction of the value obtained by the alternative estimates from the value estimated by criterion). **SD** standard deviation. **SEE** standard error of the estimate. **95% LOA** 95% limits of agreement (MD ± 1.96 x SD of differences). **LL** lower limit. **UL** upper limit. **Eq.** equivalence to criterion 4C model. **CI** confidence interval. A comparison of means between methods was performed using the paired sample t-test. Pearson's correlation (r) was performed to assess the correlation between methods and between the mean and the difference of the methods (Bland-Altman graph).

[a] Based on 5% equivalence interval, which corresponds to 1.2%

SUPPLEMENTARY MATERIAL

Rapid Four-Compartment Models in Athletes Using Alternative Solutions for Body Volume and Water

Tiago R. Silva, Rui Poínhos, Luís B Sardinha, Vitor Hugo Teixeira, Analiza M. Silva

Table S2 Comparison of FM (%) values between the criterion 4C model and alternative estimates in males.

				ES	SEE	Bland-Altman			Equivalence testing [a]	
4C model version (n = 76)	**Mean (SD)**	**MD (SD)**	**p**	**Cohen'd [95% Confidence Interval]**		**95% LOA**		**r (p)**	**Eq.**	**90% CI difference**
						LL	**UL**			
Criterion 4C model	12.4 (4.2)									
4C TBW_{BIS}	12.2 (4.3)	0.2 (1.4)	0.277	0.13 [-0.10; 0.35]	1.38	-2.6	2.9	-0.062 (0.596)	Yes	[-0.1; 0.4]
4C TBW_{Matias}	11.9 (3.0)	0.5 (2.2)	0.070	0.21 [-0.02; 0.44]	2.17	-3.9	4.9	0.555 (<0.001)	No	[-0.0; 0.9]
4C TBW_{Sun}	13.1 (3.7)	-0.7 (2.0)	0.003	-0.36 [-0.59; -0.12]	1.98	-4.5	3.1	0.263 (0.022)	No	[-1.1; -0.3]
4C $BV_{Silva}TBW_{BIS}$	12.2 (4.1)	0.2 (2.3)	0.372	0.10 [-0.12; 0.33]	2.23	-4.2	4.7	0.051 (0.660)	No	[-0.2; 0.7]
4C $BV_{Wilson}TBW_{BIS}$	16.2 (4.1)	-3.8 (2.3)	<0.001	-1.66 [-2.00; -1.31]	2.24	-8.3	0.7	0.051 (0.664)	No	[-4.2; -3.4]
4C $BV_{Heymsfield}TBW_{BIS}$	11.6 (4.1)	0.8 (2.3)	0.005	0.33 [0.10; 0.56]	2.27	-3.8	5.3	0.057 (0.622)	No	[-0.3; 1.2]
4C $BV_{Silva}TBW_{Matias}$	11.9 (2.9)	0.5 (3.1)	0.138	0.17 [-0.06; 0.40]	3.10	-5.5	6.6	0.446 (<0.001)	No	[-0.1; 1.1]
4C $BV_{Wilson}TBW_{Matias}$	15.9 (3.0)	-3.5 (3.1)	<0.001	-1.13 [-1.42; -0.84]	3.11	-9.6	2.6	0.444 (<0.001)	No	[-4.1; -2.9]
4C $BV_{Heymsfield}TBW_{Matias}$	11.3 (2.9)	1.1 (3.1)	0.004	0.34 [0.11; 0.57]	3.14	-5.1	7.2	0.446 (<0.001)	No	[-0.5; 1.7]
4C $BV_{Silva}TBW_{Sun}$	13.0 (3.7)	-0.6 (2.9)	0.059	-0.22 [-0.45; 0.01]	2.87	-6.3	5.1	0.196 (0.089)	No	[-1.2; -0.1]
4C $BV_{Wilson}TBW_{Sun}$	17.1 (3.7)	-4.7 (2.9)	<0.001	-1.60 [-1.93; -1.25]	2.89	-10.4	1.1	0.193 (0.095)	No	[-5.2; -4.1]
4C $BV_{Heymsfield}TBW_{Sun}$	12.5 (3.7)	-0.1 (2.9)	0.747	-0.04 [-0.26; 0.19]	2.90	-5.9	5.7	0.201 (0.082)	No	[-0.7; 0.5]
DXA total FM	14.4 (3.7)	-2.0 (3.5)	<0.001	-0.59 [-0.83; -0.34]	3.34	-8.9	4.8	0.165 (0.155)	No	[-2.7; -1.4]
$2C_{Siri}$	13.7 (4.2)	-1.3 (3.0)	<0.001	-0.42 [-0.66; -0.19]	2.85	-7.2	4.6	0.013 (0.910)	No	[-1.9; -0.7]
$2C_{Brozek}$	13.9 (3.9)	-1.5 (2.9)	<0.001	-0.51 [-0.75; -0.27]	2.85	-7.2	4.2	0.131 (0.259)	No	[-2.0; -0.9]

Abbreviations: **BV** body volume. **TBW** total body water. **DXA** dual-energy x-ray absorptiometry. **ES** Effect Size. **MD** mean difference (subtraction of the value obtained by the alternative estimates from the value estimated by criterion). **SD** standard deviation. **SEE** standard error of the estimate. **95% LOA** 95% limits of agreement (MD ± 1.96 x SD of differences). **LL** lower limit. **UL** upper limit. **Eq.** equivalence to criterion 4C model. **CI** confidence interval. A comparison of means between methods was performed using the paired sample t-test. Pearson's correlation (r) was performed to assess the correlation between methods and between the mean and the difference of the methods (Bland-Altman graph).

[a] Based on 5% equivalence interval, which corresponds to 0.6%

Chapter VI – Study 3

Tracking Changes in Body Composition in Athletes: Are Rapid Four-Compartment Models Valid?

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Tracking Changes in Body Composition in Athletes: Are Rapid Four-Compartment Models Valid?

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ABSTRACT

While rapid four-compartment (4C) models have demonstrated cross-sectional validity in diverse populations, including athletes, their longitudinal validity remains unverified. This study aims to evaluate the longitudinal validity of utilizing Dual-Energy X-ray Absorptiometry (DXA) derived estimates of body volume (BV) and bioelectrical impedance spectroscopy (BIS) derived total body water (TBW) in rapid 4C models in athletes. Criterion 4C used DXA for bone mineral content, air displacement plethysmography for BV, and deuterium dilution for TBW. Analyses of longitudinal changes were performed in participants who experienced changes in fat mass (FM) greater than 1% (n = 60) and in a subgroup of athletes with alterations in FM exceeding the least significant change (1.97 kg, n = 25). All alternative estimates underestimated changes compared to criterion 4C, with 4C TBW_{BIS} providing the smaller and DXA the higher mean difference (0.41 kg and 0.94 kg, respectively). Bland-Altman analyses showed wide limits of agreement (LOA) for all methods. Even the alternative with the smaller 95% LOA had a wide LOA (4C TBWBIS: -2.9% to 4.0%, n = 60). This investigation demonstrates that these rapid 4C models exhibit precision superior to or equivalent to DXA alone, while offering reduced assessment times and broader accessibility than the criterion 4C. Regarding accuracy for evaluating changes over time, despite acceptable results at the group level, interpretation should be careful at the individual level.

Key Words: athletes; body composition; dual-energy-X-ray absorptiometry; multicompartment model; validation

INTRODUCTION

Athletes frequently pursue body composition (BC) manipulation, more commonly weight loss, driven by various performance-related factors pertinent to their sport. Reducing fat mass (FM) can confer competitive advantages in certain sports, enhancing metrics such as power-to-weight ratio, movement efficiency, and diminished energy expenditure (1). Additionally, aesthetic considerations frequently serve as a motivating factor (2). While BC assessment is a standard practice in sports (3), the method should be able to accurately track changes over time for evaluating responses to dietary and/or exercise programs.

Multicompartment models, such as the four-compartment (4C) model, generally regarded as the reference method for FM (4), can account for variations in fat-free mass (FFM) characteristics (e.g., hydration), thus reducing the error in BC assessment compared to simpler two-compartment models, which rely on fixed constants (5). Indeed, not only do some populations (e.g., athletes) often deviate from these assumed constants (2), but the relative proportions of FFM components can also vary with weight fluctuations, with potential consequences for the accuracy of the simpler BC methods (6).

Despite their accuracy, multicompartment models are costly, time-consuming, and impractical for routine use (7). To overcome these limitations, a rapid 4C model was developed (8, 9) incorporating bioelectrical impedance spectroscopy (BIS) for total body water (TBW) estimation, and dual-energy x-ray absorptiometry (DXA) for body volume (BV) determination (10, 11). While the validity of rapid 4C models has been evaluated in diverse populations, including athletes (12), these studies were cross-sectional, and no study to date has evaluated the accuracy of this method to detect changes in BC over time.

Therefore, the present study aims to evaluate whether a rapid 4C model combining DXA-derived BV and BIS-derived TBW can accurately track longitudinal FM changes in athletes.

METHODS

Participants

The present study analyzed data from a previously published project (13), involving athletes competing at national and international levels in basketball, handball, volleyball, triathlon, and swimming. An observational study design was employed, with a follow-up period ranging from 5 to 10 months, depending on the competitive calendar for each sport. The initial assessment (M1) occurred at the beginning of the season, while the second assessment (M2) was conducted immediately before the main competition phase.

For this analysis, participants whose FFM hydration (TBW/FFM) values were outside the range reported (0.686 to 0.808) in nine adult human cadavers (14) were excluded (n = 5).

The study was approved by the Ethics Committee of the Faculty of Human Kinetics, University of Lisbon (Lisbon, Portugal), and was conducted according to the Declaration of Helsinki for Human Studies from the World Medical Association (15).

Body composition measurements

All BC assessments were conducted in the morning after a 12-hour overnight fast, with participants abstaining from caffeine and alcohol for the previous 24 hours and avoiding vigorous exercise for at least 15 hours prior to each measurement period (M1 and M2). The methodologies are summarised below.

Anthropometric measurements

Body weight (BW) was measured to the nearest 0.01 kg on an electronic scale connected to the plethysmograph computer (BOD POD©, COSMED, Albano Laziale, Italy), with participants wearing a bathing suit without shoes, and stature was measured to the closest

0.1 cm with a stadiometer (Seca, Hamburg, Germany). The coefficient of variation (CV) for BW, based on test-retest using ten participants, was 0.072%.

Dual-energy x-ray absorptiometry (DXA)

Whole-body DXA scans (Hologic Explorer-W, Waltman, MA, USA) were performed to estimate whole body total mass (kg), FM (kg), FFM (kg) and bone mineral content (BMC, kg), as described elsewhere (16). DXA measurement allows partitioning FFM into two components: lean soft tissue (LST) and BMC. Because BMC represents ashed bone, BMC was multiplied by 1.0436 to convert it into total bone mineral (Mo) (17). Scan positioning, acquisition, and analysis were standardized and followed the manufacturer's guidelines. For participants taller than the scan area, BC was assessed using the sum of two scans (head and trunk plus limbs) (18). The CV in our laboratory for body mass FM, LST and BMC were 1.7%, 0.8% and 1.3%, respectively (18).

DXA-derived total body volume

DXA-derived BV (DXA-BV) was determined with the following equation from Silva et al. (19):

$$\text{DXA-BV}_{\text{Silva}}\ (\text{L}) = \left(\frac{FM}{0.89}\right) + \left(\frac{LST}{1.05}\right) + \left(\frac{BMC}{7.81}\right) - 0.126 \quad \textit{(equation 1)}$$

Where FM is fat mass (kg), LST is lean soft tissue (kg) and BMC is bone mineral content (kg). The CV for $\text{DXA-BV}_{\text{Silva}}$, based on test–retest using ten participants, is 0.42%.

DXA-BV was also estimated following a theory-based approach (7), using the value for the density of LST proposed by Heymsfield and colleagues (7) (1.064 kg/L) and confirmed by our laboratory in a sample of 201 athletes (36% females) (19):

$$\textit{DXA-BV}_{\textit{Heymsfield}}\ \textit{(L)} = \left(\frac{FM}{0.9007}\right) + \left(\frac{LST}{1.064}\right) + \left(\frac{Mo}{2.982}\right) \quad \textit{(equation 2)}$$

Where FM is fat mass (kg), LST is lean soft tissue (kg) and Mo is bone mineral (kg). The CV for DXA-$BV_{Heymsfield}$, based on test–retest using ten participants, is 0.47%.

Air displacement plethysmography (ADP)

BV was determined using an air displacement plethysmograph (BOD POD® model 2007A version 5.4.3, software BOD POD Body Composition System, COSMED, Albano Laziale, Italy). Wearing a swimsuit and a swimming cap, thoracic gas volume was measured for all participants, and BV was computed by adjusting the initial BV for thoracic gas volume. Subsequently, body density was calculated as the ratio of BW to BV. The CV in our laboratory for measuring BV is 0.36% (20).

Total-body water (TBW)

TBW was measured through deuterium dilution, analyzed by a stable gas isotope ratio mass spectrometer (Hydra 20-20, PDZ, software SerCon Control Callisto v40.0.11, Europa Scientific, UK). After a 12-hour fast, an initial urine sample was collected, and a dose of deuterium oxide solution (^2H_2O) corresponding to 0.1 g/kg of BW was administered. Following a 4-hour equilibration period, a second urine sample was obtained. Our laboratory's CV for measuring TBW is 0.28% (20).

Bioimpedance analysis

BIS (model 4200 Xitron Technologies, San Diego, CA) was used as an alternative to deuterium dilution to determine TBW. BIS-derived TBW was calculated using the inherent BIS calculation based on Cole modelling (21) and mixture theories (22) rather than regression equations. A test-retest analysis of ten healthy participants showed a CV of 0.25% for BIS-derived TBW.

144

145 *Criterion Four-Compartment Body Composition Model*

146 A criterion 4-compartment model was used to assess FM, based on the equation
147 described by Wang et al. (2002) (23):

148 FM (kg)=2.748×BV-0.699×TBW+1.129×Mo – 2.051×BW *(equation 3)*

149 Where BV is body volume (L), TBW is total body water (kg), Mo is bone mineral (kg),
150 and BW is body mass (kg).

151

152 *Rapid Four-Compartment Body Composition Model*

153 Rapid 4C models were calculated using a DXA-derived value of BV as previously
154 described (equations 1 and 2) and BIS-derived TBW. Table 1 summarizes the rapid 4C models
155 evaluated.

156 *Table 1 here*

157

158 *Fat-free mass characteristics*

159 Using the abovementioned methodologies, the proportions of TBW, Mo, soft tissue
160 minerals (Ms) and protein (Prot) in FFM were estimated.

161 Ms was estimated as (24):

162 $$Ms\ (kg) = 0.0129 * TBW \quad \textit{(equation 4)}$$

163 Where TBW is total body water (L).

164 Prot was estimated as (24):

165 $$Prot\ (kg) = BW - FM - TBW - Mo - Ms \quad \textit{(equation 5)}$$

166 Where BW is body weight (kg), FM is fat mass (kg), TBW is total body water (L), Mo
167 is bone mineral (kg) and Ms is soft tissue minerals (kg).

TBW/FFM, Mo/FFM, Ms/FFM and Prot/FFM were calculated using the reference TBW estimated from deuterium dilution and the reference FFM estimated from 4C (FFM = BW – 4C FM).

The density of FFM (D_{FFM}) was estimated as (7):

$$D_{FFM}\ (kg/L) = \frac{FFM}{\frac{TBW}{0.99371} + \frac{Prot}{1.34} + \frac{Mo}{2.982} + \frac{Ms}{3.317}} \textit{ (equation 6)}$$

Where FFM refers to fat-free mass (kg), TBW is total body water (L), Prot is protein (kg), Mo is bone mineral (kg) and Ms is soft tissue minerals (kg).

Propagation of measurement error

In this study, the error associated with measuring FM from the criterion 4C model can be estimated by assuming an average BC of the whole sample (BV, TBW, Mo, and BW) and measurement precision of each method, as stated throughout this section.

$$\text{FM } \upsilon^2 = (2.748 \text{ x } 70.0 \text{ x } 0.0036)^2 + (0.699 \text{ x } 45.7 \text{ x } 0.0028)^2 + (1.129 \text{ x } 2.99 \text{ x } 0.013)^2 + (2.051 \text{ x } 74.38 \text{ x } 0.00072)^2 = 0.50 \ (\upsilon \simeq 0.71\ kg) \textit{ (equation 7)}$$

The precision of the criterion 4C model to determine FM was ≃ 0.71 kg.

Applying the same calculations for the rapid 4C models using CV% of BIS-derived TBW:

$$\textbf{4C TBW}_{\textbf{BIS}}\text{: FM } \upsilon^2 = (2.748 \text{ x } 70.0 \text{ x } 0.0036)^2 + (0.699 \text{ x } 45.3 \text{ x } 0.0025)^2 + (1.129 \text{ x } 2.99 \text{ x } 0.013)^2 + (2.051 \text{ x } 74.38 \text{ x } 0.00072)^2 = 0.50 \ (\upsilon \simeq 0.71\ kg) \textit{ (equation 8)}$$

This error propagation technique assumes that variables are independent. However, applying this method directly to the rapid 4C equations using DXA-derived BV would violate this assumption due to the interdependence between BV and Mo. DXA-derived BV is estimated using DXA outputs, including BMC, while Mo is simultaneously derived from the

same BMC (Mo = 1.034 x BMC). To resolve this multicollinearity and more accurately weight the error contribution of BMC measurement, we performed an algebraic substitution of the volume prediction equation directly into the criterion equation. This reduction consolidated the BMC terms from both the BV estimate and the Mo into a single independent term. Consequently, the measurement error was calculated by applying the previous formula to the primary independent variables (FM_{DXA}, LST_{DXA}, BMC_{DXA}, TBW, and BW) using the algebraically derived coefficients.

4C $BV_{Silva}TBW_{BIS}$: $FM\ \upsilon^2 = (3.088 \times 12.96 \times 0.017)^2 + (2.617 \times 58.04 \times 0.0077)^2 + (1.530 \times 2.86 \times 0.013)^2 + (0.699 \times 45.31 \times 0.0025)^2 + (2.051 \times 74.38 \times 0.00072)^2 = 1.85\ (\upsilon \simeq 1.36\ kg)$ *(equation 9)*

4C $BV_{Heymsfield}TBW_{BIS}$: $FM\ \upsilon^2 = (3.051 \times 12.96 \times 0.017)^2 + (2.583 \times 58.04 \times 0.0077)^2 + (2.051 \times 2.99 \times 0.013)^2 + (0.699 \times 45.31 \times 0.0025)^2 + (2.051 \times 74.38 \times 0.00072)^2 = 1.81\ (\upsilon \simeq 1.35\ kg)$ *(equation 10)*

Body composition changes analysis

Analyses of longitudinal changes were performed in participants whose FM changes exceeded the measurement error threshold for criterion 4C (≃1%) (n=60).

Given the limited research on least significant change (LSC), especially in multicompartment model studies, we performed a subgroup analysis in which LSC was defined as a value exceeding 95% prediction interval of the precision error. In accordance with the International Society for Clinical Densitometry (ISCD) protocol (25), LSC was calculated by multiplying the precision error by 2.77, yielding a minimal detectable change in FM of 1.97 kg

at a 95% confidence level. This subgroup consisted of 25 athletes whose FM alterations exceeded the LSC threshold.

Statistical analysis

IBM SPSS Statistics® (IBM, Chicago, Illinois, USA) version 29.0 for Windows® was used for data analysis. Statistical significance was set at $p < 0.05$ (two-sided). We evaluated the data distribution's normality by analyzing skewness and kurtosis, both falling within acceptable ranges of |skewness| < 3 and |kurtosis| < 8 (26).

Paired samples t-tests were performed to evaluate the differences between the criterion 4C and alternative FM estimations longitudinally (Δ = M2 – M1). The effect size (ES) of the differences was determined using Cohen's d. The magnitude of the ES was determined by Hopkin's scale (27) as follows: [0; 0.2[= trivial, [0.2; 0.6[= small, [0.6; 1.2[= moderate, [1.2; 2.0[= large, ≥ 2.0 = very large. Pearson's correlation coefficients (r) and mean differences (MD), obtained by subtracting the value obtained by alternative estimations from the value obtained by the criterion 4C, were also calculated. Positive differences indicate underestimation, while negative differences indicate overestimation. Since the interaction between sex and alternative fat mass estimations was not statistically significant ($p > 0.05$), all analyses were conducted on the whole sample.

All ΔFM estimations were converted to absolute values to compare errors between alternative FM estimations, regardless of direction.

Furthermore, we evaluated the agreement between criterion 4C and the rapid 4C estimations using the Bland-Altman method (28), including the calculation of the 95% limits of agreement (LOA). The correlations between the mean values and the differences were also determined to detect potential proportional bias. These calculations were performed using GraphPad Prism® software version 10.0.3.

242

243 **RESULTS**

244 Table 2 presents the participants' characteristics and BC at baseline (M1).

245 *Table 2 here*

246

247 *Longitudinal analysis of rapid 4C model estimates*

248 Mean DXA total body mass changes were similar to those from the electronic scale
249 connected to the plethysmograph computer (FM loss: p=0.782; FM gain: p=0.919). A total of
250 33 athletes lost FM, losing an average of -2.17 kg (SD=1.28 kg). Regarding athletes who gained
251 FM (n=27), they gained an average of 2.18 kg (SD=1.36 kg). The FFM components,
252 TBW/FFM, Prot/FFM, and Ms/FFM, significantly changed between moments (p=0.016;
253 p=0.016 and p<0.001, respectively), with no differences found for Mo/FFM and the D_{FFM}
254 (p=0.378 and p=0.161).

255 There were no differences in the errors of the alternative estimations (error = ΔCriterion
256 4C – ΔAlternative estimation) in ΔFM (kg) between the groups that lost or gained FM (p=0.165
257 to p=0.939). Also, the same was found between the sexes (p=0.183 to p=0.987).

258 Pearson correlation for changes in FM (kg) between the two visits measured by criterion
259 and alternative estimates were 0.82, 0.56, 0.56, and 0.39 (p < 0.001, n=60), for 4C TBW_{BIS}, 4C
260 $BV_{Silva}TBW_{BIS}$, 4C $BV_{Heymsfield}TBW_{BIS}$ and DXA, respectively.

261 Table 3 presents the absolute differences in ΔFM (kg and %) for each method compared
262 to the criterion. Compared to the criterion 4C model, alternative estimates consistently
263 underestimated changes, with 4C TBW_{BIS} providing the smaller and DXA the higher mean
264 difference. The Bland-Altman analysis revealed a considerable variation in agreement between
265 all methods. Even the alternative with the smaller 95% LOA, 4C TBW_{BIS}, had a wide LOA (-
266 2.9% to 4.0%).

Table 3 here

The comparison of absolute longitudinal changes of FM (kg and %) between the criterion 4C model and alternative estimates greater than the LSC can be found in Table 4. As seen in the main analysis, alternative estimates consistently underestimated the changes from 0.9% from 4C TBW_{BIS} to 2.5% from DXA. Also, the 95% LOA remained wide in this subgroup (n=25).

Table 4 here

DISCUSSION

This study provides the first longitudinal evaluation of the validity of rapid 4C models for tracking BC changes in athletes compared to a criterion 4C model using ADP and deuterium dilution. While rapid 4C models showed acceptable group-level agreement (mean difference ≈ 0.5%, corresponding to ≈20% error), their wide 95% limits of agreement (± 150%) and systematic underestimation of changes raise concerns for individual-level monitoring. These findings contrast with cross-sectional validations reporting narrower LOA (± 20%) in athletic cohorts (12), highlighting the unique challenges of longitudinal tracking of FM. The mean difference for the rapid 4C models tested was ≈ 0.5% FM, representing a mean error of approximately 20%. DXA had the highest absolute underestimation of changes (1.4% FM, approximately 50%). Although these rapid 4C models presented good results at the group level, interpretation should be careful at the individual level, as the wide LOA may limit the ability to detect biologically meaningful changes in an individual. Although previous studies (12) have shown good cross-sectional agreement with the reference model, the high variability observed in these longitudinal analyses raises concerns about the practical utility of these rapid 4C methods for detecting real changes in FM. In scenarios that require high accuracy in monitoring

variations, especially when small changes are expected, caution is recommended in adopting these techniques, and whenever possible, using the criterion model is recommended.

Despite comparable precision of TBW estimation by deuterium and BIS in our lab (CV = 0.3%), and ΔTBW_{BIS} and $\Delta TBW_{deuterium}$ not differing significantly (n=20; MD = 0.48 kg; SD = 2.79 kg; ES [95% CI] = 0.17 [-0.27; 0.61]), BIS exhibited large 95% LOA (-4.99 to 3.67 kg) for ΔTBW. Indeed, the differences in TBW changes between methods ($\Delta TBW_{deuterium}$ minus ΔTBW_{BIS}) explained 81% of the error found for 4C $BV_{Silva}TBW_{BIS}$ and, perceptibly, 100% in the case of 4C TBW_{BIS}.

While BW is typically measured with high accuracy using simple calibrated scales, determining BV requires specialized methodologies, each with inherent limitations. Underwater weighing requires subjects to be completely submerged underwater while fully exhaling their breath (29). ADP has emerged as a superior alternative due to its increased reliability and reduced invasiveness (30). Notwithstanding, ADP volume assessments can be influenced by body temperature, moisture, facial hair (e.g., the presence of facial hair can lead to an underestimation of BV due to the isothermal air trapped in the facial hair (31)), and clothing worn during testing (31, 32). Also, both methods impose determining or predicting residual lung and thoracic gas volumes (2). The intestinal and stomach gas volume, not accounted for or roughly estimated, is another methodological constraint in these techniques that can lead to an error in BV estimation (33). In contrast, DXA-derived estimates of BV are unaffected by intestinal gas since the X-ray beams do not capture them (7) and are also independent of the abovementioned limitations. Even though alternative solutions for TBW and BV have been suggested (9, 11), there is no accurate alternative for bone quantification, with DXA being essential for 4C models. Nevertheless, DXA also has recognized limitations such as participants' exposure to radiation (even if in low amounts), differences in body

315 composition assessment across various manufacturers and software versions, and the
316 underestimation of FM in lean subjects (29).

317 While measuring more components enhances validity, there is a concern about the
318 propagation of errors in determining BV, TBW, and Mo (5). Nevertheless, the improved
319 accuracy of FM estimations is not compromised (5). In this investigation, the calculated
320 propagation of error for the criterion 4C model was 0.71 kg (1%). Applying the same
321 calculations for the rapid 4C models – using CV% of DXA-derived BV and BIS-derived TBW
322 – would yield a value of 0.77 kg (1.0%), 0.82 kg (1.1%) and 0.90 kg (1.2%) for 4C TBW_{BIS},
323 4C $BV_{Silva}TBW_{BIS}$ and 4C $BV_{Heymsfield}TBW_{BIS,}$ respectively. However, for models using DXA-
324 derived BV (4C $BV_{Silva}TBW_{BIS}$ and 4C $BV_{Heymsfield}TBW_{BIS}$), this approach violates the
325 assumption of variable independence due to the interdependence between BV and Mo. By
326 applying an algebraic correction, as explained in the methods section, a more rigorous estimate
327 increased the estimated error to 1.4 kg (1.8%). While this represents a robust and conservative
328 upper bound of uncertainty, it suggests that models incorporating DXA-derived BV have lower
329 precision than the criterion. Nevertheless, we consider this an acceptable trade-off for the
330 model's enhanced efficiency, which reduces the protocol to only two devices. Crucially, this
331 practical alternative enables FM assessment without requiring athletes to move between
332 measurements, as individuals attain stable body fluid levels after undergoing the DXA scan,
333 permitting an immediate assessment in the same position using BIS. Also, the precision of
334 these rapid 4C models remains superior (4C TBW_{BIS}) or equivalent (4C $BV_{Silva}TBW_{BIS}$ and 4C
335 $BV_{Heymsfield}TBW_{BIS)}$ to the CV in our laboratory for DXA FM (1.7%).

336 Notwithstanding, the impact of this propagation of error when tracking body
337 composition changes remains unexplored. A worst-case scenario can be assumed, considering
338 independent and additive errors between the two moments. In this scenario, the impact of the

propagation error for the present study subjects would be 1.42 kg for criterion 4C, indicating that all athletes in the LSC subgroup exhibited FM changes exceeding this worst-case scenario.

Given the limited research employing the true criterion 4C model to evaluate changes in BC, particularly in athletes, some methodological caveats should be discussed and are critical to acknowledge when interpreting the results. Researchers employ multicompartment models, like the 4C model, as reference methods to assess FM in living humans (24). The 4C model improves FM estimation accuracy (5) relative to the 2C models of Siri (34) and Brozek (35) by including bone mineral and TBW, which are often treated as constant despite their fluctuations. The 4C model, while regarded as a reference method for estimating FM, is based on theoretical assumptions that may introduce limitations. Notably, this model does not consider minor components (e.g., essential lipids) and does not account for glycogen, a carbohydrate storage that can fluctuate rapidly, particularly in athletes (24). Also, multicompartment models are structured on the premise that the involved components' densities are known and constant in vivo (36). Their development employs two primary strategies: one assumes that the remaining BW, post-subtraction of FM, TBW, and Mo, comprises known-density protein and Ms (as is the case of the 4C model used in this study). Alternatively, the other approach speculates the remaining mass as a known-density combined residual mass, encompassing protein, Ms, and other components (36). Different methods generally provide comparable FM estimates due to their reliance on closely related models and assumptions (24, 36). However, despite their accuracy, these models are subject to uncertainties. Heymsfield and colleagues (24) conducted a seminal study assessing model error by evaluating how variations in model assumptions affected FM estimates in the “Reference Man”. However, no research to date has considered the implications of these errors within athletic populations.

363 Despite the fact that BC plays a significant role in athletic achievements, manipulation
364 of carbohydrate intake, including loading strategies, is a significant part of some sports and
365 crucial for optimizing performance (37), ensuring that muscle glycogen stores are sufficient to
366 meet training and competition demands (37). In athletes, glycogen is not only a rapidly
367 changing body reserve but can also represent a higher absolute value than the common
368 "Reference Man" (38). For each gram of glycogen, a minimum of three grams of water is
369 stored with it (38), so glycogen super-compensation leads to a significant increase in TBW.
370 This can be detected and quantified by dilution techniques and is likely attributed to a rise in
371 intracellular water (ICW) (39, 40). Furthermore, pertinent to our rapid 4C model, Shiose et al.
372 (39) presented the first evidence indicating that BIS has adequate sensitivity to identify
373 increases in TBW following carbohydrate loading, particularly under rigorously standardized
374 and controlled measurement conditions.
375 If the water bound to glycogen is accounted for in the TBW, omitting glycogen
376 introduces error only in the dry mass component. In our criterion model, the unaccounted dry
377 portion of glycogen is misattributed to protein, which assumes a density of 1.34 g/cm³, while
378 glycogen's density is 1.52 g/cm³ (24), hence introducing a bias. Heymsfield et al. (24) showed
379 that adding glycogen to Wang's 5C model reduced FM% by 0.6%. In our sample, the inclusion
380 of glycogen in a 6C model would give a 0.7% lower FM%. However, this 6C model assumes
381 that glycogen accounts for 4.4% of protein (24). The fixed glycogen-to-protein ratio of the 6C
382 model of 4.4% is based upon fasting-state organ-weighted calculations by Kehayias et al. (41).
383 Muscle glycogen in athletes, however, widely fluctuates with diet and training (38), making
384 this fixed constant unreliable in athletic populations. For instance, athletes can reach a high
385 muscle glycogen status only after some days on carbohydrate-rich diets, while depressed
386 glycogen stores result from prolonged and intense exercise (38).

An explicit term for essential lipids is also missing in current multicompartment models (24). Solvent extraction separates total body lipids into non-essential lipids (triglycerides, commonly referred to as "fat") and essential lipids (e.g., in the nervous system and the plasma membranes of cells (5)) (42). The commonly accepted assumption is that non-triglyceride lipids contribute only a small fraction to total body lipids and have been included with the FFM compartment (43). In rat carcasses, Comizio et al. showed that essential lipids represented 2.8% of BW (42). This percentage decreased, although not significantly, after several weeks of energy restriction, with (2.7%) or without exercise (2.2%). The authors are unaware of any available data on human cadavers but for the "Reference Man" the total FM (13.5 kg) is shown as including 1.5 kg of essential fat (≃ 2.1% BW) (44). If one assumes that the men in this study (n = 43; BW = 78.4 kg; FM = 9.9kg) have a similar quantity of essential fat (1.5 kg), this will represent a substantial proportion of total FM (≃ 15.2 % BW), thereby introducing a large error. Currently, there is no in vivo method for quantifying this compartment. However, this exclusion may lead to misinterpretation of changes, particularly if the proportion of essential lipids changes relative to other components. The only available data, from Comizio et al. (42) in rats, demonstrated that non-triglyceride lipids made up 3.3% of FFM at baseline, falling to 2.9% after 9 weeks of energy restriction alone (non-significant) and to 2.3% when exercise was included ($P = 0.01$). Further research is needed to determine how dietary and exercise interventions influence this lipid fraction.

Although the novelty of this investigation and the contribution to scientific advances, some limitations should be considered. First, on average, our sample did not lose/gain much FM, with only 25 athletes in the LSC group (mostly men, with only five being women). Second, using an older BIS device constitutes an additional limitation, and future research should study the impact of incorporating newer BIS devices in rapid 4C models. More recent BIS devices (e.g., Impedimed SFB7) have shown higher precision in the estimation of TBW than older

412 devices like the Xitron used in the present study (45). Future studies using these newer devices
413 may thus minimize errors at the individual level and reduce the error of BIS-derived TBW in
414 rapid 4C models. Thirdly, a theoretical limitation of the error propagation analysis in the rapid
415 4C using DXA-derived BV concerns the assumption of independence among DXA outputs
416 (FM, LST and BMC). At the pixel level, DXA technology relies on two-component attenuation
417 models, meaning that algorithmic misclassification of tissue creates an intrinsic negative
418 covariance between compartments (as the incorrect classification of a pixel inevitably results
419 in a simultaneous overestimation of one compartment and underestimation of the other).
420 However, manufacturer-specific covariance matrices are proprietary and unavailable.
421 Consequently, we treated the DXA outputs as independent variables in calculating the
422 propagation error. This approach is methodologically defensible for two reasons: (i) the
423 precision estimates (CV) derived from test-retest inherently capture the aggregate variability
424 caused by algorithmic misclassification; (ii) ignoring this potential negative covariance results
425 in a slightly larger estimated error, thereby providing a conservative upper bound for the
426 precision of the rapid 4C model. Finally, although the sample was small and sex-unbalanced
427 (28% females), it comprised athletes from tiers 3 (highly trained/national) and 4
428 (elite/international) (46). It is challenging to recruit this group, especially for studies involving
429 many assessments, e.g., a criterion 4C data collection requires many hours for completion. The
430 main strength of this study was the comparison against a true criterion 4C model, using ADP
431 for BV and deuterium dilution for TBW, which offered important insights into the accuracy
432 and applicability of these models.
433 In conclusion, this investigation demonstrates that these rapid 4C models exhibit
434 precision superior or equivalent to DXA alone, while offering reduced assessment times and
435 broader accessibility than the criterion 4C. Regarding accuracy for evaluating changes over

time, despite acceptable results at the group level, interpretation should be careful at the individual level.

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AUTHORS' CONTRIBUTIONS

TRS: Conceptualization, Methodology, Formal Analysis, Writing- Original draft preparation; **RP:** Formal analysis, Writing - Review & Editing; **BMPMO:** Formal analysis, Writing - Review & Editing; **DAS**: Conceptualization, Methodology, Recruitment, data collection and analysis, Writing - Review & Editing; **CNM**: Conceptualization, Methodology, Recruitment, data collection and analysis, Writing - Review & Editing; **LBS**: Writing - Review & Editing, Supervision, Funding acquisition; **VHT**: Writing - Review & Editing; **SBH**: Writing - Review & Editing; **AMS**: Conceptualization, Methodology, Writing - Review & Editing, Supervision, Funding Acquisition.

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CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

DATA AVAILABILITY

Data described in the manuscript will be made available upon reasonable request pending application and approval to analiza@fmh.ulisboa.pt.

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589

TABLES

Table 1 Variations tested in Rapid Four-Compartment Body Composition Models

Model Version	TBW	BV	Mo	BW
Criterion 4C model	Deuterium	ADP	DXA	Scale
4C TBW_{BIS}	BIS	ADP	DXA	Scale
4C $BV_{Silva}TBW_{BIS}$	BIS	DXA-BV_{Silva}	DXA	Scale
4C $BV_{Heymsfield}TBW_{BIS}$	BIS	DXA-$BV_{Heymsfield}$	DXA	Scale

Abbreviations: **4C** four-compartment, **TBW** total body water, **BV** body volume, **Mo** mineral content, **BW** body weight, **ADP** air displacement plethysmography, **BIS** bioelectrical impedance spectroscopy, **DXA** dual-energy x-ray absorptiometry

Table 2: Participants' characteristics and body composition at baseline.

	Whole sample									LSC subgroup[a]								
	Total (n=60)			Female (n=17)			Male (n=43)			Total (n=25)			Female (n=5)			Male (n=20)		
	mean	SD	Range	mean	SD	Range	mean	SD	Range	mean	SD	Range	mean	SD	Range	mean	SD	Range
Age (years)	19.5	4.2	16.0 - 31.0	16.7	0.8	16.0 - 19.0	20.6	4.5	16.0 - 31.0	20,4	5,0	16.0 - 30.0	16.2	0.4	16.0 - 17.0	21,5	5,1	16.0 - 30.0
Weight (kg)	74.4	11.7	52.9 - 102.3	64.2	6.0	52.9 - 78.0	78.4	10.9	61.4 - 102.3	78,6	12,8	54.9 - 102.3	62.6	4.6	54.9 - 66.1	82,6	10,8	68.0 - 102.3
Height (cm)	181.7	10.7	159.2 - 204.7	171.3	6.1	159.2 - 182.4	185.9	9.2	169.2 - 204.7	185,2	10,5	166.5 - 204.7	175.0	6.8	166.5 - 182.4	187,7	9,7	169.2 - 204.7
BMI (kg/m^2)	22.4	2.0	17.7 - 27.5	21.9	2.3	17.7 - 25.4	22.6	1.9	18.9 - 27.5	22,8	2,1	19.1 - 27.5	20.5	1.5	19.1 - 22.9	23,4	1,9	19.8 - 27.5
BMC (kg)	2.9	0.7	1.8 - 4.4	2.3	0.4	1.8 - 3.4	3.1	0.7	1.8 - 4.4	3,1	0,7	2.0 - 4.4	2.4	0.3	2.0 - 2.7	3,3	0,6	2.0 - 4.4
4C FM (kg)	11.3	4.6	0.8 - 20.6	14.6	3.6	8.2 - 20.6	9.9	4.3	0.8 - 20.0	11,1	4,7	0.8 - 20.0	13.3	2.9	10.1 - 16.8	10,6	4,9	0.8 - 20.0
4C FM (%)	15.4	6.5	1.1 - 30.5	22.7	4.9	15.2 - 30.5	12.5	4.6	1.1 - 23.4	14,3	6,1	1.1 - 28.7	21.4	5.5	15.2 - 28.7	12,6	4,9	1.1 - 21.1
4C FFM (kg)	63.1	11.8	39.1 - 84.3	49.6	5.1	39.1 - 62.0	68.5	9.0	52.7 - 84.3	67,5	12,1	39.1 - 84.3	49.3	6.3	39.1 - 56.0	72,0	8,3	58.1 - 84.3

Data is presented as mean (SD).

Abbreviations: **LSC** least significant change, **SD** standard deviation, **BMI** body mass index, **BMC** bone mineral content, **FM** fat-mass, **FFM** fat-free mass, **4C** four-compartment. [a]LSC was defined by a value that exceeded the 95% prediction interval of the precision error (calculated by multiplying the precision error by 2.77), which corresponds to 1.97 kg

Table 3 Comparison of absolute longitudinal changes of FM (kg and %) between the criterion 4C model and alternative estimates (n=60).

				ES	Bland-Altman		
					95% LOA		r (p)
Method	**Mean (SD)**	**MD (SD)**	**p**	**Cohen' d [95% Confidence Interval]**	**Lower**	**Upper**	
FM (kg)							
Criterion 4C model	2.17 (1.31)						
4C TBW_{BIS}	1.76 (1.49)	0.41 (1.33)	0.019	0.31 [0.05; 0.57]	-2.19	3.01	-0.156 (0.235)
4C $BV_{Silva}TBW_{BIS}$	1.69 (1.53)	0.48 (1.68)	0.031	0.31 [0.05; 0.56]	-2.82	3.78	-0.161 (0.219)
4C $BV_{Heymsfield}TBW_{BIS}$	1.67 (1.51)	0.50 (1.68)	0.024	0.30 [0.04; 0.56]	-2.78	3.78	-0.151 (0.249)
DXA total FM	1.23 (1.36)	0.94 (1.53)	<0.001	0.62 [0.34; 0.89]	-2.05	3.93	0.356 (0.005)
FM (%)							
Criterion 4C model	2.7 (1.7)						
4C TBW_{BIS}	2.2 (2.1)	0.5 (1.8)	0.026	0.29 [0.03; 0.55]	-2.9	4.0	-0.256 (0.048)
4C $BV_{Silva}TBW_{BIS}$	2.2 (2.1)	0.5 (2.2)	0.057	0.25 [-0.08; 0.51]	-3.7	4.8	-0.219 (0.093)
4C $BV_{Heymsfield}TBW_{BIS}$	2.2 (2.1)	0.6 (2.1)	0.049	0.26 [0.00; 0.52]	-3.6	4.8	-0.213 (0.102)
DXA total FM	1.3 (1.1)	1.4 (1.9)	<0.001	0.70 [0.42; 0.98]	-2.4	5.2	0.434 (0.001)

Abbreviations: **BV** body volume. **TBW** total body water. **DXA** dual-energy x-ray absorptiometry. **ES** Effect Size. **MD** mean difference (subtraction of the value obtained by the alternative estimates from the value estimated by criterion). **SD** standard deviation. **95% LOA** 95% limits of agreement (MD ± 1.96 x SD of differences). **Δ** longitudinal changes (obtained by subtracting values at the second assessment [M2] from the initial assessment [M1], Δ = M2 – M1). A comparison of means between methods was performed using the paired sample t-test. Pearson's correlation (r) was performed to assess the correlation between methods and between the mean and the difference of the methods (Bland-Altman plot). All ΔFM (kg and %) estimations were converted to absolute values to compare errors between alternative FM estimations, regardless of direction.

Table 4 Comparison of absolute longitudinal changes of FM (kg and %), between the criterion 4C model and alternative estimates, greater than the least significant change (n=25).

				ES	Bland-Altman		
					95% LOA		r (p)
Method	**Mean (SD)**	**MD (SD)**	**p**	**Cohen' d [95% Confidence Interval]**	**Lower**	**Upper**	
FM (kg)							
Criterion 4C model	3.26 (1.37)						
4C TBW_{BIS}	2.58 (1.64)	0.69 (1.69)	0.060	0.41 [-0.01; 0.81]	-2.63	4.00	-0.190 (0.364)
4C $BV_{Silva}TBW_{BIS}$	2.21 (1.97)	1.05 (2.26)	0.029	0.46 [0.05; 0.87]	-3.38	5.49	-0.350 (0.087)
4C $BV_{Heymsfield}TBW_{BIS}$	2.18 (1.96)	1.09 (2.25)	0.024	0.48 [0.06; 0.89]	-3.33	5.49	-0.345 (0.092)
DXA total FM	1.37 (1.13)	1.89 (1.81)	<0.001	1.04 [0.55; 1.52]	-1.66	5.43	0.192 (0.358)
FM (%)							
Criterion 4C model	4.0 (1.9)						
4C TBW_{BIS}	3.2 (2.3)	0.9 (2.1)	0.050	0.41 [0.00; 0.82]	-3.3	5.0	-0.242 (0.245)
4C $BV_{Silva}TBW_{BIS}$	2.8 (2.6)	1.3 (2.8)	0.038	0.44 [0.03; 0.85]	-4.3	6.8	-0.325 (0.114)
4C $BV_{Heymsfield}TBW_{BIS}$	2.8 (2.6)	1.3 (2.8)	0.034	0.45 [0.04; 0.86]	-4.3	6.8	-0.321 (0.117)
DXA total FM	1.5 (1.2)	2.5 (2.3)	<0.001	1.11 [0.60; 1.60]	-2.0	7.1	0.402 (0.046)

Abbreviations: **BV** body volume. **TBW** total body water. **DXA** dual-energy x-ray absorptiometry. **ES** Effect Size. **MD** mean difference (subtraction of the value obtained by the alternative estimates from the value estimated by criterion). **SD** standard deviation. **95% LOA** 95% limits of agreement (MD ± 1.96 x SD of differences). **Δ** longitudinal changes (obtained by subtracting values at the second assessment [M2] from the initial assessment [M1], $\Delta = M2 - M1$). A comparison of means between methods was performed using the paired sample t-test. Pearson's correlation (r) was performed to assess the correlation between methods and between the mean and the difference of the methods (Bland-Altman plot). All ΔFM (kg and %) estimations were converted to absolute values to compare errors between alternative FM estimations, regardless of direction. Least significant change was defined by a value that exceeded the 95% prediction interval of the precision error (calculated by multiplying the precision error by 2.77), which corresponds to 1.97 kg.

Chapter VII – Study 4

Examining Intra- and Inter-Tester Reliability of Post-Scan Dual-Energy X-ray Absorptiometry Regional Body Composition Analysis in Athletes

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Examining Intra- and Inter-Tester Reliability of Post-Scan Dual-Energy X-ray Absorptiometry Regional Body Composition Analysis in Athletes

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ABSTRACT

Background/objectives: The purpose of this investigation was i) to assess the intra- and inter-tester reliability of delineating regions of interest (ROIs) in Dual-energy X-ray Absorptiometry (DXA) for measuring regional fat mass (FM), lean soft tissue (LST), and bone mineral content (BMC) and ii) to investigate the prevalence of inconsistencies between automatically defined ROIs and manufacturer guidelines. A secondary aim was to assess whether discrepancies in the delineation of ROIs by different testers could bias regional body composition changes over time. **Methods**: DXA measurements were performed with a total body scan in 86 athletes (64.0% females) from 12 sports. Two independent testers conducted post-scan analysis following the manufacturer's guidelines. Intra- and inter-tester reliability was evaluated using the intra-class correlation coefficient (ICC), mean differences, paired samples t-tests and Bland-Altman plots. **Results:** Intra- and inter-tester reliability was nearly perfect (ICC: 0.998-1.000) across all regions and components (FM, LST, and BMC). Mean differences were consistently <1% for all regions and components, except for left arm FM (1.4%; SD=4.6%) in inter-tester reliability. Inconsistencies between automatically defined ROIs and manufacturer guidelines were frequent, with chest line corrections (which separate the arm from the trunk) required in 91.9% of scans. Inter-tester ICC for regional changes (n=52) ranged from 0.668 to 0.981, with wider limits of agreement than cross-sectional estimates. **Conclusion:** DXA post-scan regional analysis can be performed with very high reliability and low measurement error, but manual review is essential. When monitoring changes over time, baseline and follow-up scans should be analyzed by the same evaluator.

Key Words: Body composition; Dual-energy X-ray Absorptiometry; Manual analysis; Precision; Reliability;

INTRODUCTION

The utilization of Dual-energy X-ray Absorptiometry (DXA) to assess body composition has been considerably more widespread in recent years, most likely driven by increased equipment availability in both research and field settings (1, 2). An international follow-up survey on body composition practices in sports reported an 11% increase in the use of DXA from 2013 to 2022, highlighting its growing importance in the field (3). DXA offers several advantages for physique assessment in athletes, as it is a fast, non-invasive, and safe method with low-radiation exposure, which enables the evaluation of regional body composition, including lean soft tissue (LST), fat mass (FM), and bone mineral content (BMC) (1).

Most seasonal changes in an athlete's body composition are modest (4, 5). Precision must be taken into account when attempting to differentiate between true meaningful change and the inherent error of the measurement process (6). The precision of DXA measurements is highly dependent on a comprehensive multi-step procedure for executing a whole-body scan (7). This procedure involves three crucial phases: subject preparation, accurate positioning, and post-processing of the scan data (7). Although most focus is generally on subject presentation (1) and consistent patient positioning (8), careful post-scan analysis is also essential for minimizing measurement errors (9).

Most DXA software automatically defines regional body composition areas, such as of left and right arms, legs, and trunk. Still, despite this automatic placement of regions of interest (ROIs) for body subregions, it's typically necessary to manually revise these automated placements (10). Ensuring consistency in placement using bony landmarks is crucial for enhancing the reproducibility of results within each subregion (9). Libber et al. (2012) (11) reported that manual analysis alterations were almost always required, with the head cut line demanding the most frequent adjustment (39 out of 40 cases).

Prior research has predominantly focused on comparing automatically and manually defined ROIs (10, 11). Yet, there is a notable gap in assessing the reproducibility of ROI measurements in DXA scans, particularly regarding intra- and inter-measurer reliability. This gap is further compounded by the limited investigations being conducted on older Lunar (12) or Hologic (13) devices, with no examination to date of the latest versions of DXA equipment featuring more advanced software.

Therefore, our primary objectives were to (i) assess the intra-tester and inter-tester reliability of delineating ROIs in DXA for measuring regional FM, LST, and BMC and (ii) to investigate the prevalence of inconsistencies between automatically defined ROIs and manufacturer guidelines. Moreover, a secondary aim was to assess whether discrepancies in the delineation of ROIs by different testers could bias regional body composition changes over time.

METHODS

Participants

The following inclusion criteria were adopted: (i) Trained/developmental (or higher) athletes, which corresponds to tier 2 or higher according to the classification framework of McKay et al. (14), and (ii) having at least one year of specific sport training. On the other hand, exclusion criteria included (1) taking any medications or having any clinical condition known to affect body water or composition and (2) pregnancy. A sample size of 78 participants was needed to estimate an intraclass correlation coefficient (ICC) of 0.90 with a 95% confidence interval width of 0.1 and 80% assurance probability (15).

The baseline sample consisted of 86 athletes (64.0% females) spanning various sports: rhythmic gymnastics (n=37 females), soccer (n=17; 14 females), CrossFit (n=10; 9 males), rugby (n=7 males), road running (n=4; 3 males), canoeing (n=3; 2 males), Hyrox (n=3 males),

and basketball, taekwondo, cycling, and jiu-jitsu (n=1 male each), and weightlifting (n=1 female). Our sample was composed of 33.7% of tier 2 (Trained/Developmental), 57.0% of tier 3 (Highly Trained/National Level) and 9.3% of tier 4 (Elite/International Level) athletes.

The study was approved by the Ethics Committee of the Faculty of Nutrition and Food Sciences of the University of Porto (FCNAUP, 153/2023/CEFCNAUP) and was conducted according to the Declaration of Helsinki for Human Studies from the World Medical Association (16). Written informed consent to participate was received from all participants or parents/legal guardians before testing.

Procedures

Most athletes were evaluated during the months of October 2024 to December 2024 for the first moment (M1) and during the months of March to May 2025 for the second moment (M2). Regarding female gymnasts' data collection, M1 occurred before the beginning of the competitive period (December 2023) and M2 occurred about a week after the conclusion of the competitive season. Some gymnasts ended their season in March 2024 because they did not qualify for nationals and were evaluated there, while the rest were reevaluated in April 2024.

Body composition measurements

BC assessments were conducted in the morning (between 8 am and 12 pm) after an overnight fast of at least 8 hours, and an indication to avoid moderate to vigorous exercise in the previous 12 hours. Additionally, participants were advised to maintain regular water intake and avoid dehydration-promoting environments (e.g., saunas) in the 24 hours preceding the scan.

Anthropometric measurements

Participants were weighed to the nearest 0.1 kg with a scale (Seca 876, Hamburg, Germany) and stature was measured to the closest 0.1 cm with a stadiometer (Seca, Hamburg, Germany).

Dual-energy x-ray absorptiometry

DXA measurements were performed with a total body scan Hologic Horizon Wi, Hologic APEX software version 5.6.1.3 (Horizon Wi, Hologic, Waltham, MA, USA). The equipment was calibrated each morning before measurements, following the manufacturer's instructions, and was consistently operated by the same technician. The precision was evaluated according to the International Society for Clinical Densitometry (ISCD) guidelines (17), with duplicate DXA measurements being taken in 30 independent recreationally active individuals (mean age = 26.1 years, SD = 6.8; mean weight = 66.1 kg, SD = 12.2; mean height = 169.9 cm, SD = 8.5). Full repositioning occurred between measurements, as participants were requested to leave the scanning table and get onto it once again for each acquisition. This resulted in a coefficient of variation (CV) for FM, LST and BMC of 1.6%, 0.6% and 1.1%, respectively. These values fall within the acceptable precision outlined in the official position statement by the ISCD (18). Supplementary Table 1 reports the CV for each assessed region and compartment.

Participants were asked to remove metallic objects (e.g., earrings, rings…), and if their clothing or underwear had metal attachments, they were given a hospital gown. To ensure consistent and reproducible positioning during DXA scans, the position protocol of head, arms, hands and legs based on the National Health and Nutrition Examination Survey was followed (19), as recommended by ISCD (18). Participants were manually assisted into a supine position centred on the scanner table. The head, neck, and torso were aligned parallel to the long axis of the scan bed, while the shoulders and pelvis were positioned perpendicular to it. Both arms

were placed by their sides with palms resting on the examination table, and legs were positioned at shoulder width with a 45° internal rotation. The arms were positioned to create maximum space between them and the trunk and legs, ensuring clear separation and facilitating posterior analysis. This arrangement allows for the distinct delineation of the arms from the rest of the body during the scan analysis. The feet were secured together using velcro strips to minimize incidental movement.

Scan analysis

The scan analysis was conducted using Hologic APEX software version 5.6.1.3. Based on the manufacturer's guidelines, the regional delimitation lines were reviewed and adjusted as necessary to correct any inaccuracies in the automatic placement. Specifically, the sub-region analysis tool was used to draw customized ROIs, capturing five segments: the right arm, left arm, right leg, left leg, and trunk. The boundaries used to identify these segments are detailed in Figure 1.

Figure 1

To ensure blinding during scan analysis, the DXA software's "auto-analyze" function was employed before each evaluation. This feature automatically resets the placement of ROI lines, ensuring that each tester begins their assessment with the default, machine-generated lines without access to the adjustments made in the previous analysis.

Autoanalysis Evaluation

To investigate the prevalence of inconsistencies between automatically defined ROIs and manufacturer guidelines, the initial analysis of the scans was conducted using the automatic analysis feature provided by the software. Following this, the technician reviewed and adjusted the placement of regional analysis lines following the manufacturer's guidelines, making

corrections when clear inaccuracies were identified. Adjustments were specifically guided by the protocol outlined in Figure 1. During manual analysis, tools including "line mode", "point mode" and the “brightness/contrast” adjustment located on the left panel of the analysis interface were utilized. The "line mode" allowed the repositioning of an entire line, while the "point mode" enabled the precise movement of a specific point along a line. Brightness and contrast adjustments were employed to enhance the visibility of anatomical features, ensuring optimal alignment with the manufacturer's specifications. Each adjustment to a line or a specific point along a line was recorded as a manual modification of the lines detailed in Figure 1.

Testers

Two testers were involved in the study. Tester 1, responsible for intra-tester reliability, positioned the participants, performed the scans, executed the initial analysis and determined the prevalence of inconsistencies between automatically defined ROIs and manufacturer guidelines. Tester 1 had prior experience with DXA analysis and completed a comprehensive 16-hour training provided by Hologic. Tester 2 was a trainee nutritionist who received supervised training and practice from Tester 1 and was also provided with the manufacturer's user guide. Prior to this study, Tester 2 had no previous experience with DXA scan analysis.

To evaluate intra-tester reliability, Tester 1 re-analysed all scans (n=86). To prevent recall bias, a minimum of one month’s separation was maintained between the two assessments. However, given the two-year data collection period, this was significantly longer in many cases. For inter-tester reliability, Tester 1 and Tester 2 each performed a single, independent analysis of the same scans. Both testers were blinded to each other’s results.

Regional body composition changes

198 Fifty-two athletes had the necessary data for the secondary objective, which aimed to
199 investigate whether discrepancies in regional body composition changes over time arise when
200 different testers are used to delineate ROIs. Supplementary Table 2 presents the participants'
201 characteristics for this sub-sample. Most participants were female gymnasts (n=52, 71%). For
202 this analysis, both testers analysed all athletes' scans at the two moments, and differences were
203 compared between testers.
204
205 **Statistical analysis**
206 IBM SPSS Statistics® (IBM, Chicago, Illinois, USA) version 29.0 for Windows® was
207 used for data analysis. Statistical significance was set at $p < 0.05$ (two-sided). Data were
208 reported as mean (standard deviation [SD]). Normality was evaluated by skewness and
209 kurtosis. The values of skewness and kurtosis were within acceptable ranges if $|skewness| < 3$
210 and $|kurtosis| < 8$ (20).
211 Paired samples t-tests were performed to evaluate the differences between scan
212 analyses. The effect size (ES) of the differences was determined using Cohen's d. The
213 magnitude of the ES was determined by Hopkin's scale (21) as follows: [0; 0.2[= trivial, [0.2;
214 0.6[= small, [0.6; 1.2[= moderate, [1.2; 2.0[= large, ≥ 2.0 = very large. Measurement error
215 was quantified as absolute differences (kg) and relative differences (%). Absolute error
216 reflected differences in body component masses within the same tester (intra-tester error) or
217 between testers (inter-tester error, calculated as the difference between the second tester's
218 measurements and the mean of the two assessments by tester 1). The relative error for tester 1
219 was calculated as the difference in body component masses divided by its mean mass for each
220 segment. In contrast, inter-measurer relative differences were determined as the absolute
221 difference divided by the mean of tester 1's two assessments for each segment. Intraclass
222 correlation coefficients (ICCs) were employed to assess both within-measurer and between-

tester reliability (two-way random effects, absolute agreement, single rater/measurement) (22). Furthermore, we evaluated the agreement between scan analyses using the Bland-Altman method (23), including the calculation of the 95% limits of agreement (LOA). This analysis also included the analysis of the correlation (r, Pearson correlation coefficient) between the mean values and the differences to detect potential proportional bias. These calculations were performed using GraphPad Prism® software version 10.0.3.

Paired samples t-tests (or Wilcoxon signed-rank tests for non-normally distributed data – arms FM) were used to compare the percentage error between the arms and legs for each component (FM, LST, BMC). Independent samples t-tests were used to assess differences in manual corrections between sexes, while one-way ANOVA was applied to evaluate differences across sports.

RESULTS

Table 1 presents the sample's characteristics at baseline (M1). Participants exhibited a weight range from 22.8 kg to 105.7 kg, with an average body fat percentage of 21.9% (SD = 5.3), ranging from 10.2% to 38.3%. Participant height ranged from 123.5 cm to 193.9 cm, and the mean age was 21.5 years (SD = 10.5), ranging from 8 to 50 years. They had a mean training age of 10.1 years (SD = 7.2), ranging from 1 to 39 years.

Prevalence of inconsistencies between automatically defined ROIs and manufacturer guidelines

The frequency of manual corrections during analysis is presented in Table 2. Each scan required an average of 2.5 manual line corrections (SD = 1.2), with the number of corrections ranging from none to a maximum of 5 out of the 8 lines evaluated. The chest lines exhibited the highest frequency of manual corrections, with adjustments needed in 91.9% of scans,

248 followed by the T12-L1 line (41.9%) and the upper pelvic line (33.7%). In contrast, the leg
249 lines required no corrections, and the leg divider line had the lowest adjustment frequency
250 among altered lines (9.3%). The number of corrections did not differ significantly between
251 males and females (males: 2.2 [SD = 1.2]; females: 2.6 [SD = 1.2], p = 0.175) or between sports
252 (p = 0.444).
253
254 Intra-tester reliability
255 The ICC, mean differences (in kg or %), effect size, and 95% LOA (in kg or %) of the
256 intra-tester reliability are provided in Table 3. Tester 1 demonstrated nearly perfect intra-tester
257 reliability across all five regions and components (FM, LST, and BMC), with ICC values
258 ranging from 0.998 to 1.000. Percentage mean differences were consistently below 1% for all
259 regions and components. While most comparisons showed no statistically significant
260 differences between repeated analyses, significant differences with small effect sizes were
261 observed for left arm LST (p = 0.030, ES [95% CI] = 0.2 [0.0; 0.5]), right arm FM (p = 0.016,
262 ES [95% CI] = 0.3 [0.1; 0.5]), and right arm LST (p = 0.016, ES [95% CI] = 0.3 [0.0; 0.5]).
263 The Bland-Altman analysis showed small limits of agreement overall, either in kg or
264 %, but notable variability was observed in the left arm FM%, where the 95% LOA ranged from
265 -17.5% to 16.0%. Pearson's correlation coefficient between the mean and differences for
266 regional body components was not statistically significant for most regions, except for the left
267 arm FM (r = 0.267, p = 0.013), left arm LST (r = 0.224, p = 0.038), left arm BMC (r = 0.265,
268 p = 0.014), right arm LST and BMC (r = 0.315, p = 0.003 and r = 0.256, p = 0.017, respectively)
269 and trunk FM (r = -0.246, p = 0.023), indicating some weak, but significant, proportional bias
270 in these specific regions.
271 A significant difference in relative error for FM was observed between legs and arms
272 (p = 0.007), with greater relative errors in arms. Arms also showed significantly higher relative

error for LST values than legs ($p = 0.014$), while no significant difference was found for BMC ($p = 0.279$).

Inter-tester reliability

Regarding inter-tester reliability, the ICC, mean differences (in kg or %), effect size, and 95% LOA (in kg or %) are provided in Table 4. It was found to have nearly perfect inter-tester reliability across all five regions and components (FM, LST, and BMC), with ICC values ranging from 0.998 to 1.000. Percentage mean differences were consistently below 1% for all regions and components, except for left arm FM (MD = 1.4%; SD = 4.6%). While most comparisons showed statistically significant differences, all effect sizes were small.

The Bland-Altman analysis, either in kg or %, showed small limits of agreement overall, except for left arm FM (95% LOA -10.0% to 7.3%) and right arm FM (95% LOA -9.6% to 10.2%). Pearson's correlation coefficient between the mean and differences for FM was statistically significant for most regions, indicating weak (ranging from -0.478 to 0.331) but significant proportional bias.

A significant difference in relative error for FM was observed between legs and arms ($p = 0.002$), with greater relative errors in arms. No significant differences were found for LST ($p = 0.075$) and BMC ($p=0.749$).

Inter-tester reliability - Regional body composition changes

Regarding inter-tester reliability for regional body composition changes, the ICC, mean differences (in kg or %), effect size, and 95% LOA (in kg or %) are provided in Table 5. ICC values ranged from 0.668 to 0.981. The Bland-Altman analysis showed wider limits of agreement than what was cross-sectionally found for inter-tester reliability.

298 **DISCUSSION**

299 This study investigated the intra- and inter-tester reliability of DXA-derived regional
300 body composition in athletes, focusing specifically on the post-processing stage of the multi-
301 step whole-body DXA procedure. The current findings showed that post-scan DXA regional
302 body composition analysis can be reliably performed both within and between testers, with
303 very low measurement error and very high agreement (< 1% mean difference; ICC = 0.998-
304 1.000). Furthermore, results highlight the prevalence of inconsistencies between automatically
305 defined ROIs and manufacturer guidelines, reinforcing the necessity for manual corrections
306 across different anatomical regions in athletes, particularly for certain lines, such as the chest
307 and spine, which require more frequent adjustments.

308 The number of corrections needed in auto-analyzed scans and the observed
309 inconsistencies suggest that technicians should manually analyze all athletes' DXA scans for
310 the best accuracy and precision. Similarly, Baniak et al. (24) concluded that manually derived
311 bone mineral density and t-scores were more accurate than auto-analysis. Also, our results
312 confirm a pattern consistently reported in the literature, i.e. a higher relative error in arms than
313 in legs (25, 26). This difference likely stems from the overlap between trunk and arm region,
314 causing some inconsistencies in delineating arm ROI across subjects and testers, which is
315 particularly aggravated in more muscular individuals. In our sample, the FFM index was
316 positively associated with inter-tester percentage error, explaining 20% of the variance in arm
317 LST and 18% in arm BMC, respectively. Supporting this finding, it was found that chest lines
318 (which separate the arm from the trunk) required manual correction in 91.9% of scans. In
319 addition, as Rothney stated (26), relatively small absolute changes can result in
320 disproportionately large percentage changes, magnifying the apparent error.

321 Overall, the results confirm excellent agreement for FM measurements, except for the
322 left arm, where higher variability was primarily due to an outlier, an 8-year-old female

gymnast, whose left arm FM differed between scan analyses by only -0.049 kg (0.086 kg in the first scan and 0.039 kg in the second) in intra-tester reliability analysis. Despite the small absolute difference, this translated into a large percentage change of 74.8%. The 95% LOA without this participant were -5.2% to 5.5%, similar to what was found in other regions. The gymnast was intentionally retained in the analysis to reflect the potential for significant variability in percentage error in specific contexts, such as lightweight athletes with very small amounts of FM.

Translational research yields the greatest practical value when its design and implementation closely mirror the real-world context it aims to inform. DXA utilization in sport has been considerably more widespread in recent years (3), and all operators performing DXA scanning should be properly trained by the manufacturer (27). While manufacturers usually give several hours of formal hands-on training during the initial purchase of a system (27), this may not always be possible for all operators. Our study suggests that, when official training is not possible, supervised training and practice with an experienced tester and adherence to the manufacturer´s guidelines can yield reliable outcomes. This finding goes along with the existing literature (28), which indicates that extensive experience, while valuable, is not necessarily imperative for producing reliable results. However, these positive results are partially attributed to careful positioning of the subjects (18), which facilitated posterior post-scan analysis, and a highly standardized protocol for drawing ROIs. This includes positioning the arms to create maximum separation from the trunk and legs, and asking female athletes to remove objects from behind the head or ponytails to prevent forward displacement of the chin, which complicates accurate head line placement (11).

In research, particularly in multicenter studies, multiple investigators may perform the scans, making post-scan reliability particularly important. Our results indicate excellent cross-sectional group-level reliability. However, in applied and clinical contexts, where individual

longitudinal monitoring is the focus, scans should ideally be analyzed by the same evaluator at baseline and follow-up. In our dataset, for example, a baseline ALST of 22.4 kg obtained by one tester could vary by -0.670 kg to +0.373 kg if analyzed by another tester. This inter-tester variability adds to the already known patient-positioning variability (18) and subject presentation (1), further reducing the precision to detect true meaningful changes. Indeed, the 95% limits of agreement for ALST across testers for regional body composition changes (n = 52) were wide (-4.2% to 9.5%).

Moreover, assessing the precision of a tester, as suggested by ISCD (17), is crucial when the goal is to monitor and interpret body composition changes over time to evaluate the impact of training programs and/or nutrition interventions (29). Indeed, in our dataset, only 57.7% of the athletes add ALST changes larger than the least significant change (LSC). In other words, just over half (58%) of the athletes demonstrated "true" changes in ALST with 95% confidence, highlighting the need for careful interpretation in practical scenarios.

One of the major strengths of this study is the deliberate isolation of the post-scan analysis step, which enabled the assessment of its specific contribution to measurement variability independent of subject position and preparation. Another strength of the primary objectives is the heterogeneity of the sample, which included athletes across different sports and levels of competition. The differences among participants increase the generalizability of the ecological reliability in regard to age, body mass, height, FM, and numerous athletic profiles. Nonetheless, several limitations should be acknowledged. First, the underage group was composed almost exclusively of rhythmic gymnasts. As a result, any stratification by age would be inherently confounded by sport-specific morphological characteristics, preventing meaningful interpretation of potential developmental effects. Future research including younger athletes from a broader range of sports is necessary to specifically examine the influence of skeletal maturation on ROI delineation. Second, the subgroup reassessed

longitudinally was disproportionately female (77%), with a large proportion of gymnasts (~70%), which poses a limitation on generalizing these findings. Moreover, findings are specific to a single DXA manufacturer and model, and may not fully apply to other systems.

In conclusion, this study demonstrates that DXA regional body composition post-scan analysis can be performed with very high reliability and low measurement error both within and between testers. However, manual review using a standardized protocol is essential to minimize variability, especially in arm ROIs where inconsistencies are common. Furthermore, when monitoring body composition changes over time, baseline and follow-up scans should be analyzed by the same evaluator to ensure precision in detecting true change.

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AUTHORS' CONTRIBUTIONS

TRS: Conceptualization, Methodology, Data Collection, Formal Analysis, Writing-Original draft preparation, **BG**: Data Collection, Writing - Review & Editing, **RP**: Conceptualization, Methodology, Formal Analysis, Writing - Review & Editing, Supervision. **VHT**: Writing - Review & Editing, Supervision. **AMS:** Conceptualization, Methodology, Writing - Review & Editing, Supervision.

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CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

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485 **FIGURE LEGENDS**

486 **Figure 1:** Graphical representation of anatomical boundaries and placement of regional
487 analysis lines for segmental body composition analysis, following the manufacturer's
488 guidelines.

489

TABLES

Table 1 Participants' age, anthropometric characteristics and body composition.

	Total (n = 86)		Female (n = 55)		Male (n = 31)	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
Age (years)	21.1	10.5	15.6	6.8	30.7	9.0
Weight (kg)	60.1	22.2	47.3	15.3	82.7	12.3
Height (cm)	162.4	17.4	152.9	14.2	179.1	6.8
BMI (kg/m²)	21.8	4.5	19.6	3.5	25.8	3.3
FM (kg)	12.6	5.0	11.6	4.9	14.5	4.9
FM (%)	21.9	5.3	24.4	4.1	17.4	4.1
LST (kg)	44.4	18.0	33.1	10.3	64.5	8.5
BMC (kg)	2.4	0.9	1.9	0.7	3.3	0.5
ALST (kg)	22.4	9.1	16.8	5.5	32.3	4.8
LARM FM (kg)	0.7	0.3	0.6	0.3	0.7	0.3
LARM LST (kg)	2.4	1.3	1.6	0.5	3.9	0.8
LARM BMC (kg)	0.1	0.1	0.1	0.0	0.2	0.0
RARM FM (kg)	0.7	0.3	0.6	0.3	0.8	0.3
RARM LST (kg)	2.5	1.3	1.7	0.5	3.9	0.8
RARM BMC (kg)	0.2	0.1	0.1	0.0	0.2	0.0
LLEG FM (kg)	2.6	0.9	2.5	1.0	2.6	0.9
LLEG LST (kg)	10.1	3.7	8.1	2.7	13.6	2.1
LLEG BMC (kg)	0.4	0.2	0.3	0.1	0.6	0.1
RLEG FM (kg)	2.6	1.0	2.5	1.0	2.7	0.9
RLEG LST (kg)	7.5	3.1	5.5	1.9	10.9	1.5
RLEG BMC (kg)	0.4	0.2	0.3	0.1	0.6	0.1
Trunk FM (kg)	5.2	2.7	4.4	2.3	6.5	2.7
Trunk LST (kg)	21.6	8.9	16.1	5.4	31.4	4.2
Trunk BMC (kg)	0.7	0.3	0.5	0.2	0.9	0.2

Abbreviations: **SD** standard deviation, **BMI** body mass index, **FM** fat mass, **LST** lean soft tissue, **BMC** bone mineral content, **ALST** appendicular lean soft tissue, **LARM** left arm, **RARM** right arm, **LLEG** left leg, **RLEG** right leg

497 **Table 2**. Frequency of manual corrections during analysis.

498

	Line	Manual correction n (%)
1	Neck line	17 (19.8)
2	T12-L1 line	36 (41.9)
3	Upper pelvic line	29 (33.7)
4	Lower pelvic divider lines	25 (29.1)
5	Chest lines	79 (91.9)
6	Spine lines	17 (19.8)
7	Leg lines	0 (0.0)
8	Leg divider line	8 (9.3)

Table 3 Intra-tester reliability for FM, LST, and BMC across the five segmental regions.

Regions (n = 86)	ICC	MD (SD), kg	MD (SD), %	p	ES	Bland-Altman (kg)			Bland-Altman (%)	
					Cohen' d [95% Confidence Interval]	95% LOA		r (p)	95% LOA	
						LL	UL		LL	UL
Appendicular lean soft tissue	0.999	-0.001 (0.403)	-0.1 (1.4)	0.976	0.00 [-0.22; 0.21]	-0.792	0.789	0.128 (0.240)	-3.0	2.7
Left arm FM	0.999	0.002 (0.016)	-0.8 (8.5)	0.190	0.14 [-0.07; 0.35]	-0.029	0.033	0.267 (0.013)*	-17.5	16.0
Left arm LST	0.999	0.014 (0.060)*	0.5 (2.0)	0.030	0.24 [0.02; 0.45]	-0.104	0.132	0.224 (0.038)*	-3.5	4.5
Left arm BMC	0.999	0.000 (0.003)	0.1 (1.6)	0.114	0.17 [-0.04; 0.39]	-0.005	0.006	0.265 (0.014)*	-3.1	3.3
Right arm FM	0.999	0.004 (0.014)*	0.5 (2.7)	0.016	0.27 [0.05; 0.48]	-0.023	0.030	0.115 (0.294)	-4.7	5.8
Right arm LST	0.999	0.017 (0.065)*	0.2 (2.6)	0.016	0.27 [0.05; 0.48]	-0.111	0.146	0.315 (0.003)*	-4.8	5.3
Right arm BMC	0.999	0.001 (0.003)	0.0 (1.9)	0.068	0.20 [-0.02; 0.41]	-0.005	0.006	0.256 (0.017)*	-3.7	3.8
Left leg FM	0.998	-0.005 (0.055)	-0.3 (1.9)	0.420	-0.09 [-0.30; 0.13]	-0.112	0.102	0.105 (0.338)	-4.0	3.4
Left leg LST	0.998	-0.018 (0.214)	-0.3 (1.8)	0.429	-0.09 [-0.30; 0.13]	-0.439	0.402	0.065 (0.554)	-3.9	3.3
Left leg BMC	1.000	0.000 (0.004)	-0.1 (0.7)	0.473	-0.08 [-0.29; 0.13]	-0.008	0.007	0.045 (0.678)	-1.4	1.2
Right leg FM	0.998	-0.011 (0.067)	-0.4 (2.1)	0.116	-0.17 [-0.38; 0.04]	-0.142	0.119	-0.087 (0.425)	-4.5	3.7
Right leg LST	0.998	-0.015 (0.196)	-0.2 (2.0)	0.493	-0.07 [-0.29; 0.14]	-0.398	0.369	-0.020 (0.852)	-4.0	3.6
Right leg BMC	1.000	0.000 (0.004)	-0.1 (0.7)	0.419	-0.09 [-0.30; 0.12]	-0.008	0.007	-0.029 (0.788)	-1.4	1.2
Trunk FM	0.998	0.004 (0.148)	0.3 (2.6)	0.810	0.03 [-0.19; 0.24]	-0.286	0.294	-0.246 (0.023)*	-4.7	5.4
Trunk LST	0.999	-0.003 (0.348)	0.1 (1.3)	0.926	-0.01 [-0.22; 0.20]	-0.685	0.679	-0.128 (0.241)	-2.5	2.7
Trunk BMC	0.999	0.000 (0.010)	0.1 (1.7)	0.991	0.00 [-0.21; 0.21]	-0.023	0.022	-0.051 (0.639)	-3.9	4.1

Abbreviations: **FM** fat mass. **LST** lean soft tissue. **BMC** bone mineral content. **ICC** Intraclass correlation coefficient. **MD** mean difference. **SD** standard deviation. **ES** Effect Size. **95% LOA** 95% limits of agreement (MD ± 1.96 x SD of differences). **LL** lower limit. **UL** upper limit. A comparison of means between analysis was performed using the paired samples t-test. Pearson's correlation (r) was performed to assess the correlation between analysis and between the mean and the difference of the analysis (Bland-Altman graph). *Significant change ($p < 0.05$).

Table 4 Inter-tester reliability for FM, LST, and BMC across the five segmental regions.

					ES	Bland-Altman (kg)			Bland-Altman (%)	
Regions (n = 86)	**ICC**	**MD (SD), kg**	**MD (SD), %**	**p**	**Cohen' d [95% Confidence Interval]**	**95% LOA**		**r (p)**	**95% LOA**	
						LL	**UL**		**LL**	**UL**
Appendicular lean soft tissue	0.999	-0.149 (0.266)**	-0.5 (0.9)	<0.001	-0.56 [-0.79; -0.33]	-0.670	0.373	-0.478 (<0.001)**	-2.3	1.2
Left arm FM	0.999	-0.005 (0.016)*	-1.4 (4.6)	0.002	-0.34 [-0.56; -0.12]	-0.036	0.025	-0.095 (0.383)	-10.0	7.3
Left arm LST	0.999	-0.015 (0.057)*	-0.5 (1.9)	0.019	-0.26 [-0.47; -0.04]	-0.127	0.098	-0.318 (0.003)*	-4.1	3.3
Left arm BMC	0.999	-0.001 (0.003)*	-0.5 (1.6)	0.012	-0.28 [-0.49; -0.06]	-0.008	0.006	-0.155 (0.154)	-7.2	5.3
Right arm FM	0.998	-0.002 (0.018)	0.2 (4.4)	0.423	-0.09 [-0.30; 0.13]	-0.038	0.034	-0.339 (0.001)*	-9.6	10.2
Right arm LST	0.999	-0.026 (0.050)**	-0.9 (1.7)	<0.001	-0.52 [-0.74; -0.29]	-0.123	0.073	-0.408 (<0.001)**	-4.2	2.5
Right arm BMC	1.000	-0.001 (0.002)**	-0.6 (1.3)	<0.001	-0.51 [-0.73; -0.28]	-0.008	0.006	-0.241 (0.025)*	-6.4	5.3
Left leg FM	0.999	-0.016 (0.037)**	-0.6 (1.3)	<0.001	-0.43 [-0.65; -0.21]	-0.089	0.056	-0.293 (0.006)*	-3.0	1.9
Left leg LST	0.999	-0.064 (0.150)**	-0.5 (1.2)	<0.001	-0.43 [-0.65; -0.20]	-0.357	0.229	-0.350 (0.001)*	-2.9	1.9
Left leg BMC	1.000	-0.001 (0.003)*	-0.1 (0.5)	0.015	-0.27 [-0.48; -0.05]	-0.008	0.007	-0.201 (0.064)	-2.2	2.1
Right leg FM	0.999	-0.015 (0.043)*	-0.5 (1.5)	0.002	-0.34 [-0.56; -0.13]	-0.099	0.069	-0.313 (0.003)*	-3.5	2.5
Right leg LST	0.999	-0.045 (0.121)**	-0.5 (1.3)	<0.001	-0.37 [-0.59; -0.15]	-0.281	0.191	-0.356 (0.001)*	-3.0	2.1
Right leg BMC	1.000	-0.001 (0.002)*	-0.2 (0.4)	0.002	-0.35 [-0.57; -0.13]	-0.008	0.006	-0.046 (0.671)	-2.0	1.6
Trunk FM	0.999	0.023 (0.103)*	0.3 (1.8)	0.044	0.22 [0.01; 0.43]	-0.179	0.224	0.287 (0.007)*	-3.3	3.9
Trunk LST	1.000	0.090 (0.236)**	0.3 (0.9)	<0.001	0.38 [0.16; 0.60]	-0.372	0.552	0.331 (0.002)*	-1.4	2.0
Trunk BMC	0.999	0.003 (0.010)*	0.4 (1.3)	0.004	0.32 [0.10; 0.54]	-0.019	0.026	0.087 (0.426)	-2.7	3.9

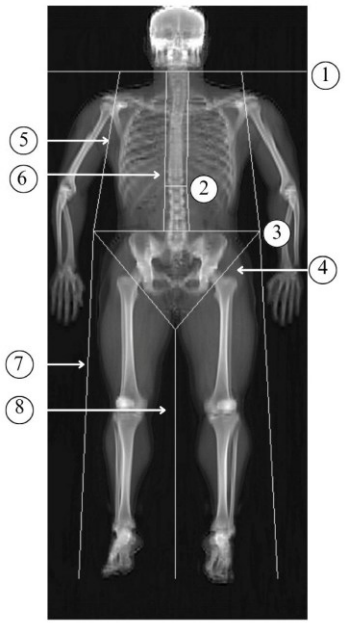
Abbreviations: **FM** fat mass. **LST** lean soft tissue. **BMC** bone mineral content. **ICC** Intraclass correlation coefficient. **MD** mean difference. **SD** standard deviation. **ES** Effect Size. **95% LOA** 95% limits of agreement (MD ± 1.96 x SD of differences). **LL** lower limit. **UL** upper limit. A comparison of means between testers was performed using the paired samples t-test. Pearson's correlation (r) was performed to assess the correlation between testers and between the mean and the difference of the testers (Bland-Altman graph). *Significant change ($p < 0.05$). **Significant change ($p < 0.001$).

Table 5 Inter-tester reliability for regional body composition changes for FM, LST, and BMC across the five segmental regions.

Regions (n = 52)	ICC	MD (SD), kg	MD (SD), %[a]	p	ES	Bland-Altman (kg)			Bland-Altman (%)	
					Cohen' d [95% Confidence Interval]	95% LOA		r (p)	95% LOA	
						LL	UL		LL	UL
ΔAppendicular lean soft tissue	0.782	0.049 (0.440)	-0.1 (1.6)	0.426	0.11 [-0.16; 0.38]	-0.81	0.91	0.106 (0.453)	-4.2	9.5
ΔLeft arm FM	0.955	0.004 (0.021)	1.1 (4.4)	0.199	0.18 [-0.09; 0.45]	-0.04	0.05	-0.038 (0.790)	-6.6	16.1
ΔLeft arm LST	0.668	0.014 (0.095)	0.3 (3.2)	0.288	0.15 [-0.13; 0.42]	-0.17	0.20	0.315 (0.023)*	-8.5	15.6
ΔLeft arm BMC	0.708	0.001 (0.004)	0.2 (2.5)	0.318	0.14 [-0.13; 0.41]	-0.01	0.01	0.240 (0.087)	-8.1	14.3
ΔRight arm FM	0.968	-0.001 (0.021)	-0.5 (4.9)	0.721	-0.05 [-0.32; 0.22]	-0.04	0.04	0.107 (0.453)	-7.2	14.4
ΔRight arm LST	0.845	0.023 (0.075)*	0.5 (2.6)	0.032	0.31 [0.03; 0.58]	-0.13	0.17	-0.081 (0.565)	-5.9	13.0
ΔRight arm BMC	0.833	0.001 (0.003)*	0.1 (2.1)	0.048	0.28 [0.00; 0.56]	-0.01	0.01	0.122 (0.389)	-5.1	11.8
ΔLeft leg FM	0.930	0.003 (0.070)	-0.1 (2.5)	0.734	0.05 [-0.23; 0.32]	-0.13	0.14	0.180 (0.201)	-5.6	14.4
ΔLeft leg LST	0.740	0.021 (0.242)	-0.1 (2.3)	0.528	0.09 [-0.19; 0.36]	-0.45	0.50	0.113 (0.425)	-5.2	12.7
ΔLeft leg BMC	0.969	0.001 (0.005)	0.0 (1.0)	0.344	0.13 [-0.14; 0.41]	-0.01	0.01	0.102 (0.472)	-3.5	6.8
ΔRight leg FM	0.947	-0.014 (0.068)	-0.7 (2.4)	0.141	-0.21 [-0.48; 0.07]	-0.15	0.12	-0.080 (0.573)	-6.1	12.2
ΔRight leg LST	0.696	-0.009 (0.187)	-0.4 (2.1)	0.719	-0.05 [-0.32; 0.22]	-0.38	0.36	0.100 (0.480)	-5.9	11.0
ΔRight leg BMC	0.981	0.000 (0.003)	-0.2 (0.7)	0.608	-0.07 [-0.34; 0.20]	-0.01	0.01	0.004 (0.979)	-2.3	5.0
ΔTrunk FM	0.975	0.023 (0.122)	0.7 (2.7)	0.173	0.19 [-0.08; 0.47]	-0.22	0.26	0.208 (0.139)	-4.9	11.1
ΔTrunk LST	0.869	-0.055 (0.428)	0.1 (1.7)	0.362	-0.13 [-0.40; 0.15]	-0.89	0.78	0.322 (0.020)*	-4.8	8.4
ΔTrunk BMC	0.868	0.001 (0.011)	0.1 (1.8)	0.748	0.05 [-0.23; 0.32]	-0.02	0.02	-0.110 (0.436)	-3.8	7.2

Abbreviations: **FM** fat mass. **LST** lean soft tissue. **BMC** bone mineral content. **ICC** Intraclass correlation coefficient. **MD** mean difference. **SD** standard deviation. **ES** Effect Size. **95% LOA** 95% limits of agreement (MD ± 1.96 x SD of differences). **LL** lower limit. **UL** upper limit. A comparison of means between testers was performed using the paired samples t-test. Pearson's correlation (r) was performed to assess the correlation between testers and between the mean and the difference of the testers (Bland-Altman graph). *Significant change ($p < 0.05$).

[a] inter-tester relative differences of the differences were determined as the absolute difference of the differences divided by the mean of tester 1's two assessments in M1 for each segment.



	Line	Description
1	Neck line	First horizontal line, separating the head from the rest of the body. Underneath the mandibular (not touching).
2	T12-L1 line	Separates the lumbar from the thoracic spine. On the disk space between T12 and L1.
3	Upper pelvic line	Touches the superior part of the iliac crest.
4	Lower pelvic divider lines	Separates the legs and trunk. Bisects both femoral necks.
5	Chest lines	Separates the arm from the trunk. The upper end is positioned between the head of the humerus and the scapula at the glenoid fossa.
6	Spine lines	Close to the spine, matching the curvature if possible.
7	Leg lines	Two lower outer lines. Separate the legs from the arms.
8	Leg divider line	Separates both legs.

SUPPLEMENTARY MATERIAL

Examining Intra- and Inter-Tester Reliability of Post-Scan Dual-Energy X-ray Absorptiometry Regional Body Composition Analysis in Athletes

Table S1 Precision and least significant change for the various body composition regions and compartments

	TEM[a] (kg)	LSC_{TEM}[b] (kg)	CV[a] (%)	$LSC_{CV\%}$[b] (%)
FM	0.243	0.673	1.6	4.4
LST	0.269	0.745	0.6	1.7
BMC	0.027	0.075	1.1	3.0
ALST	0.216	0.598	0.9	2.5
ARM FM	0.034	0.094	4.2	11.6
ARM LST	0.056	0.155	2.1	5.8
ARM BMC	0.003	0.008	2.1	5.8
LEG FM	0.092	0.255	2.8	7.8
LEG LST	0.126	0.349	1.3	3.6
LEG BMC	0.010	0.028	2.1	5.8
Trunk FM	0.208	0.576	3.4	9.4
Trunk LST	0.189	0.524	0.8	2.2
Trunk BMC	0.014	0.039	2.0	5.5

Abbreviations: **TEM** technical error of measurement, **CV** coefficient of variation, **LSC_{TEM}** least significant change for the technical error of measurement, **$LSC_{CV\%}$** least significant change for the coefficient of variation, **FM** fat mass, **LST** lean soft tissue, **BMC** bone mineral content, **ALST** appendicular lean soft tissue. The CV for arms and legs was calculated using the mean of the right and left sides.

[a]Precision was evaluated with duplicate DXA measurements in 30 individuals. Full repositioning occurred between measurements.

[b]Least significant change (LSC) was defined by a value that exceeded the 95% prediction interval of the precision error (calculated by multiplying the precision error by 2.77)

SUPPLEMENTARY MATERIAL

Examining Intra- and Inter-Tester Reliability of Post-Scan Dual-Energy X-ray Absorptiometry Regional Body Composition Analysis in Athletes

Table S2 Participants' characteristics and body composition for regional body composition changes analysis.

	Total (n = 52)		**Female (n = 40)**		**Male (n = 12)**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
Age (years)	16.9	9.6	12.8	5.3	30.7	7.9
Weight (kg)	52.3	23.8	41.7	13.7	87.8	13.1
Height (cm)	155.6	18.6	147.9	13.3	181.0	7.7
BMI (kg/m²)	20.4	4.9	18.5	3.3	26.8	3.8
FM (kg)	11.3	4.8	10.1	4.1	15.4	4.8
FM (%)	22.6	4.8	24.1	4.0	17.4	3.3
LST (kg)	38.3	18.9	29.3	9.3	68.2	8.3
BMC (kg)	2.0	1.0	1.6	0.5	3.6	0.5
ALST (kg)	19.3	9.6	14.8	5.0	34.3	4.7
LARM FM (kg)	0.6	0.3	0.5	0.3	0.8	0.3
LARM LST (kg)	2.0	1.3	1.4	0.5	4.1	0.8
LARM BMC (kg)	0.1	0.1	0.1	0.0	0.2	0.0
RARM FM (kg)	0.6	0.3	0.5	0.2	0.8	0.3
RARM LST (kg)	2.1	1.3	1.5	0.5	4.2	0.8
RARM BMC (kg)	0.1	0.1	0.1	0.0	0.3	0.1
LLEG FM (kg)	2.4	1.0	2.2	0.9	2.9	1.0
LLEG LST (kg)	8.8	3.9	7.1	2.5	14.4	2.1
LLEG BMC (kg)	0.4	0.2	0.3	0.1	0.7	0.1
RLEG FM (kg)	2.4	1.0	2.2	0.9	3.0	1.1
RLEG LST (kg)	6.4	3.3	4.8	1.6	11.6	1.2
RLEG BMC (kg)	0.4	0.2	0.3	0.1	0.7	0.1
Trunk FM (kg)	4.4	2.4	3.7	1.8	6.9	2.4
Trunk LST (kg)	18.5	9.4	14.1	4.9	33.1	4.4
Trunk BMC (kg)	0.6	0.3	0.4	0.2	1.0	0.2

Abbreviations: **SD** standard deviation, **BMI** body mass index, **FM** fat mass, **LST** lean soft tissue, **BMC** bone mineral content, **ALST** appendicular lean soft tissue, **LARM** left arm, **RARM** right arm, **LLEG** left leg, **RLEG** right leg

Chapter VIII – General discussion

The general purpose of the present dissertation was to increase the knowledge about molecular-level body composition assessment in athletes. The primary emphasis was on rapid 4C models using alternative solutions for BV and TBW. At the same time, the precision of a less commonly examined step of the multi-step procedure for executing a whole-body DXA scan, the post-scan analysis, was also studied.

General overview

Previously, body composition assessment was available only at a small number of universities and medical research centers[1]. This reality has changed, and the assessment of body composition is now routinely implemented not only in research but also across applied sports contexts, including clubs, federations, and other sports organizations[2]. Body composition research has been driven by technological advances, and, undoubtedly, there will continue to be a demand for easier, less expensive, and more practical methods[1]. Evaluating the accuracy of existing and emerging technologies is essential to ensure they are successful in guiding clinical decisions and enhancing athletic outcomes[3].

The proposed rapid 4C model, with alternative solutions for BV and TBW presented in **studies 2** (Chapter V) and **3** (Chapter VI), meets several practical considerations outlined by Bennet and Lim (2025)[3] when evaluating body composition in research and clinical practice. Compared to the criterion 4C model, this alternative is less costly, easier to use, and requires less expertise with considerably shorter preparation, data collection, and analysis times. Additionally, it demonstrated higher accuracy than DXA[4], which is commonly used as a reference when criterion 4C is unavailable. Indeed, most validation studies on smartphone camera estimations, smartwatch-based BIA, and 3D scanners have used DXA as the reference methodology[5].

With **studies 1, 2 and 3** (Chapters IV to VI), the author and his colleagues have confirmed the superior accuracy of rapid multi-compartment models compared to 2C and 3C models (DXA) in athletes, suggesting their potential to replace DXA as the criterion in body composition evaluation, where implementing the true criterion 4C model is impractical. In more depth, the appropriateness of pre-existing DXA-derived BV estimates in athletes was addressed for the first time, leading to the development of a novel equation tailored to this population. Additionally, a pioneering comparative analysis of both empirical and theoretical methods for estimating BV from DXA was conducted. Specifically, the validity of a theory-based volume

estimation approach in athletes was evaluated using a newly established LST density value for this population, thereby filling a critical gap in the literature. Lastly, the validity of different rapid multicompartment models using alternative solutions for BV and TBW was addressed for the first time in athletes.

In addition to its essential role in 4C models, DXA enables the assessment of regional body composition, offering potential utility for identifying performance-affecting asymmetries and guiding post-injury rehabilitation[6, 7]. A whole-body DXA scan encompasses subject preparation, careful positioning, and post-processing of the scan data[8]. Although the methodological literature frequently emphasizes subject presentation[9] and consistent patient positioning[10], the impact of careful post-scan analysis to maximize the precision[11] of regional body composition estimates remained underexplored. Thus, in **study 4** (Chapter VII), the intra-tester and inter-tester reliability of delineating ROIs in DXA for measuring regional FM, LST, and BMC was assessed, and the prevalence of inconsistencies between automatically defined ROIs and manufacturer guidelines was evaluated.

Study 4 (Chapter VII) highlighted that personnel using DXA should manually analyze all athletes' scans and have a well-defined protocol for the placement of regional analysis lines for segmental body composition to maintain consistency in the post-scan analysis, particularly in the arms ROIs. Additionally, although the findings demonstrate that DXA regional body composition post-scan analysis can be reliably performed within and between testers, with low measurement error and high agreement, the baseline and follow-up assessments should be conducted by the same evaluator when the goal is to monitor changes over time.

Overall, this dissertation and the aforementioned studies make a substantial contribution to the literature on molecular-level body composition assessment in athletes. Collectively, these four studies advance knowledge on the accuracy and practicality of these assessments, with both methodological and practical implications that reflect the evolution from methods to applications. This research successfully translates methodological refinements (such as the development of an athlete-specific DXA-derived BV equation and the first estimate of lean soft-tissue density in athletes) into more accessible and faster multicompartment models for evaluating athletic populations. Additionally, it reinforced the critical need for standardized manual review of post-scan DXA analysis for regional body composition assessment.

Figure 2.1 shows a schematic overview of the studies comprising this dissertation and their interrelationships.

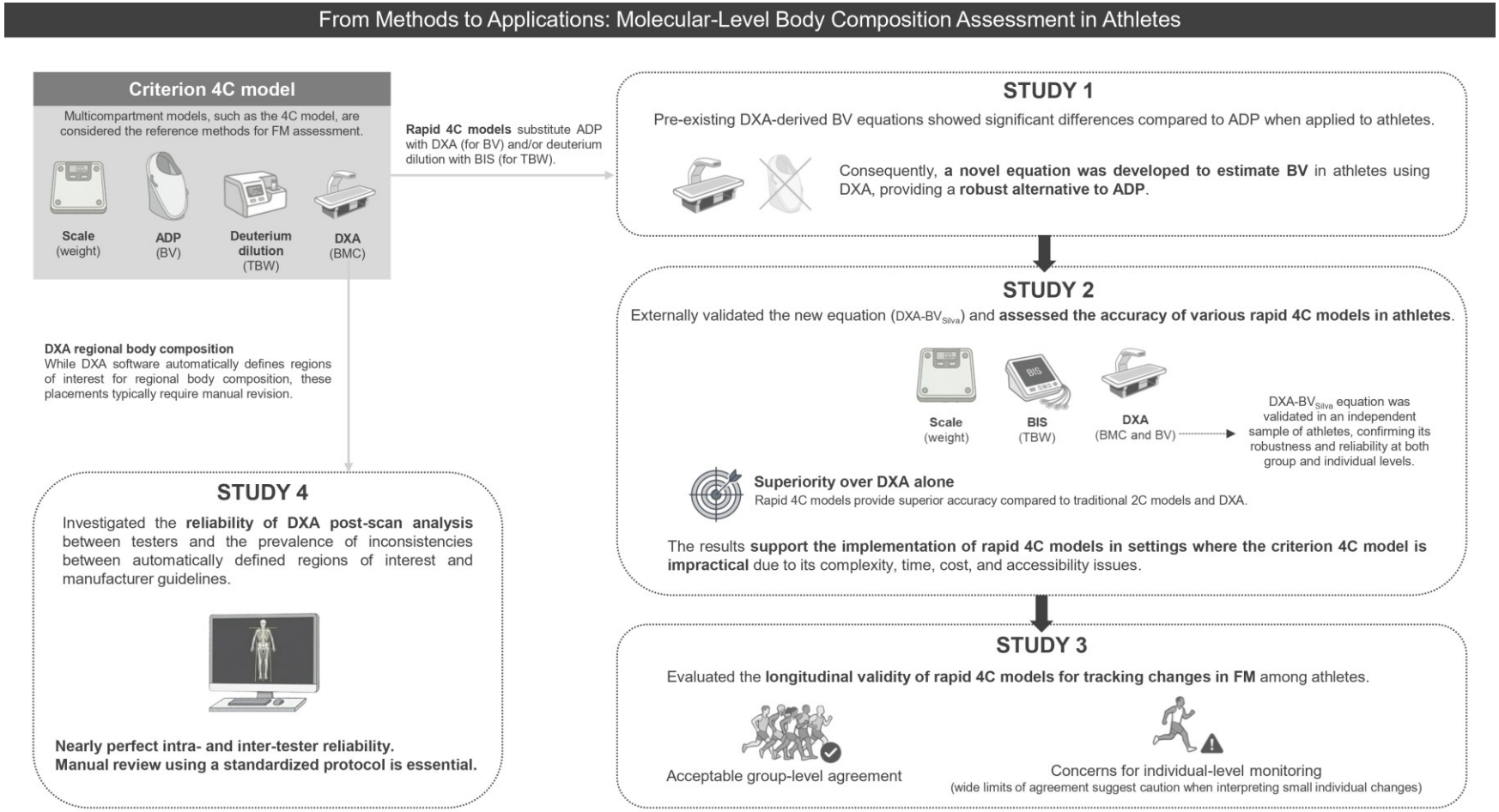


Figure 2.1 Schematic overview of the studies comprising this dissertation.

Main research findings

In summary, the main findings of this dissertation are outlined below for each article.

- Study 1 (Chapter IV):
    a. This study found that pre-existing DXA-derived BV equations showed significant mean differences and wide 95% limits of agreement when applied to an athletic sample. This confirms that empirical DXA-BV models are population-specific, especially for athletes who have distinct body composition characteristics.
    b. The author and his colleagues developed (n = 232) and validated (n = 100) a novel DXA-derived BV equation, DXA-BV_{Silva}, specifically for athletes. It provided the closest BV estimate to the criterion (ADP), with a negligible mean difference (0.05 L, SD = 0.46 L) and narrower 95% limits of agreement (-0.86 to 0.96 L) compared to those of existing equations.
    c. Additionally, lean soft tissue density was estimated for the first time in athletes at 1.064 kg/L (SD = 0.006) from a subset of 201 participants. The calculated value of 1.064 kg/L aligns with the provisional value suggested by Heymsfield et al. (2021)[12] for non-athletic samples. The theory-based approach for BV estimation (which uses this newly estimated value) also showed good agreement with ADP, particularly when incorporating Mo instead of BMC, which slightly improved its predictive capability.
    d. In conclusion, this study has contributed to the field by developing and validating a DXA-derived BV equation, expanding upon the groundwork conducted by Wilson et al. (2013)[13] and enabling BV estimation in athletes, particularly when ADP or UWW is impractical or unavailable.

- Study 2 (Chapter V):
    a. This study externally validated the DXA-BV_{Silva} equation in an independent sample of athletes, confirming its robustness and reliability at both group and individual levels.
    b. The main aim was to assess the accuracy of rapid 4C models that replaced deuterium dilution with BIS for TBW estimation and used DXA-derived BV. To address this, deuterium dilution was employed as a criterion to measure TBW, enabling a thorough evaluation of alternative methods for both BV and TBW.

c. Among the various rapid 4C models tested, 4C TBW_{BIS} (using ADP for BV and BIS for TBW), 4C $BV_{Silva}TBW_{BIS}$ (using DXA-BV_{Silva} for BV and BIS for TBW), and 4C $BV_{Heymsfield}TBW_{BIS}$ (using Heymsfield's theory-based approach for BV and BIS for TBW) provided the most accurate FM estimates. These models showed small, non-significant differences to the criterion 4C model (4C TBW_{BIS}: mean difference = - 0.18 kg, SD = 1.25 kg; 4C $BV_{Silva}TBW_{BIS}$: mean difference = - 0.23 kg, SD = 1.82 kg; 4C $BV_{Heymsfield}TBW_{BIS}$: mean difference = 0.18 kg, SD = 1.87 kg). 4C TBW_{BIS} consistently displayed the narrowest 95% limits of agreement for FM differences across the whole sample, females, and males. Among rapid 4C models using DXA-derived BV, 4C $BV_{Silva}TBW_{BIS}$ showed the narrowest 95% limits of agreement.
d. The rapid 4C models demonstrated superior accuracy in FM estimation compared to traditional 2C models (e.g., $2C_{Siri}$ and $2C_{Brozek}$) and 3C models (DXA alone). DXA alone, for instance, showed notably wide 95% limits of agreement (- 6.12 to 3.29 kg) and a mean overestimation of approximately 1.5 kg in FM compared to the criterion 4C model. This is particularly notable given the abundance of validation studies comparing alternative methods to DXA.
e. These results support the implementation of rapid 4C models in settings where the criterion 4C model (requiring ADP, deuterium dilution, and DXA) is impractical due to its complexity, time, cost, and accessibility issues. This rapid 4C model offers reduced time and equipment requirements, making it particularly attractive for athletes with tight schedules and rigorous training routines.

- <u>Study 3 (Chapter VI)</u>:
  a. While rapid 4C models have demonstrated cross-sectional validity across diverse populations, including athletes, their longitudinal validity has not been verified. Study 3 aimed to evaluate the longitudinal validity of utilizing DXA-derived estimates of BV and BIS-derived TBW in rapid 4C models in athletes.
  b. All tested alternative methods systematically underestimated longitudinal FM changes. Among these, 4C TBW_{BIS} (using ADP for BV and BIS for TBW) demonstrated the smallest mean difference (0.41 kg), whereas DXA alone showed the highest underestimation (0.94 kg).

c. Although the rapid 4C models showed acceptable group-level agreement, the 95% limits of agreement remained wide across all methods (e.g., 4C TBWBIS: -2.9% to 4.0%, n = 60), suggesting that interpreting FM changes at the individual level must be done with caution.
d. Regarding precision, rapid 4C models exhibited precision superior to or equivalent to that of DXA alone, while offering the practical advantages of reduced assessment time and broader accessibility compared with the criterion 4C model.

- Study 4 (Chapter VII):
  a. This study investigated the intra- and inter-tester reliability of DXA-derived regional body composition in athletes, focusing specifically on the post-processing stage of the multi-step whole-body DXA procedure.
  b. DXA measurements were performed with a total body scan in 86 athletes (64.0% females) from 12 sports. Two independent testers conducted a post-scan analysis in accordance with the manufacturer's guidelines. Intra- and inter-tester reliability was evaluated using the intra-class correlation coefficient, mean differences, paired samples t-tests, and Bland-Altman plots.
  c. This study showed that post-scan DXA regional body composition analysis can be reliably performed both within and between testers, with very low measurement error and very high agreement (mean difference <1%; ICC = 0.998-1.000).
  d. Furthermore, the results highlight the prevalence of inconsistencies between automatically defined ROIs and manufacturer guidelines, reinforcing the necessity for manual corrections across different anatomical regions in athletes, particularly for specific lines, such as the chest, which require more frequent adjustments (91.9%). Manual review, conducted using a standardized protocol, is crucial for minimizing variability.
  e. When monitoring body composition changes over time, the same evaluator should analyze baseline and follow-up scans to ensure precision in detecting true change.

General strengths and limitations

While each study's strengths and limitations are discussed in their respective chapters, this section synthesizes the collective strengths and limitations to provide a more comprehensive perspective on the contributions and constraints.

A significant strength of this research is its large sample size, comprising 561 athletes from various sports and competitive levels across the four studies. Recruiting this group is particularly challenging, especially given the logistical burden and time-intensive nature of the criterion 4C model (studies 2 and 3), which includes protocols such as deuterium dilution. Moreover, the BV prediction equation was developed using a considerably larger sample (n = 232) than any existing empirical equation, thereby reducing each participant's influence on the developed coefficients. Moreover, for the first time in the literature, lean soft-tissue density was estimated in a large athletic sample (n = 201). Additionally, the external validation of the DXA-derived BV equation in an independent sample enhances the robustness and reliability of our findings. The BV equation, developed and validated in Study 1, was externally validated in an independent sample in Study 2, in line with the TRIPOD recommendations[14]. Overall, the use of state-of-the-art methodologies represents a key strength of this work.

General limitations should also be discussed. Across the first three studies, samples were exclusively composed of athletes aged 16 years and older. When available, Tanner Stage V was used based on self-assessment. For participants without Tanner data, it was assumed that those 16 or older were at Stage V, as most adolescents reach the final stage (postpubertal, Stage V) around 15.6 years old in boys and 15.8 years old in girls; however, individual timing may vary[15]. This approach helped to reduce the variability in body composition associated with pubertal development. Additionally, the ages of athletes span from 16 to 38 years old, with the 95th percentile being 30 years, indicating that most participants (from studies 1, 2, and 3) were relatively young. This limitation restricts generalization for adolescents and master athletes. Moreover, our third study sample did not show significant changes in FM, with only 25 athletes exceeding the least significant change (primarily men). Lastly, it is advisable to perform repeated body composition measurements within the same menstrual cycle phase; however, the menstrual cycle phase was not taken into consideration.

Practical implications and future research

Potential directions for future research in the field of athlete body composition assessment could be derived from the findings of this dissertation:

- A 2024 systematic review by Serafini et al.[16] showed that most anthropometric equations were developed using DXA as the reference, with only one study using a 4C model[17]. These rapid 4C models will enable more research groups to create predictive equations for field practitioners (mostly anthropometric, as this is the most widely used method[2]) based on a more robust reference method. Researchers without access to ADP or deuterium dilution techniques can now develop additional criterion-derived equations tailored to specific sports and competition levels, thereby addressing gaps in the literature. As this rapid 4C model requires less time and equipment, it is beneficial for athletes with tight schedules and rigorous training routines. Consequently, these methods may also mitigate the issue of small sample sizes prevalent in the current literature of predictive equations in athletes.
- Although study 3 offered critical insights by validating rapid 4C models for tracking changes in FM, this validation is limited by the fact that only 25 athletes exceeded the least significant change (mostly men). Future research should delve deeper into the validity of rapid 4C models for tracking changes in body composition over time using larger, sex-balanced athletic cohorts whose changes in FM exceed the least significant change.
- Additionally, the methodological caveats of the criterion 4C model discussed in study 3 (do not consider minor components [e.g., essential lipids] and do not account for glycogen, a carbohydrate storage that can fluctuate rapidly, particularly in athletes) should be further discussed and explored. Moreover, further research is needed to determine how dietary and exercise interventions influence the essential lipid fraction.
- Further investigation, including younger athletes from a broader range of sports, is warranted to specifically examine the influence of age and skeletal maturation on ROI delineation in DXA post-scan analysis.

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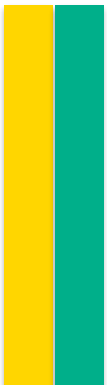

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