UNIVERSIDADE TÉCNICA DE LISBOA

FACULDADE DE MOTRICIDADE HUMANA

Thigh Composition, Cardiorespiratory Fitness and Physical Activity in a Weight Loss Program with Overweight and Obese Women

Dissertação apresentada com vista à obtenção do grau de Doutor no Ramo de Motricidade Humana, Especialidade de Saúde e Condição Física

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Para:

Zé Luís, Guilherme e Inês

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General Abstract

The purpose of the present Thesis was to analyze thigh composition through body composition methods and alterations research areas and its associations with cardiorespiratory fitness (CRF) and physical activity (PA) in overweight and obese premenopausal women. Three studies were conducted within the PESO program (Promotion of Execise and Health in Obesity), a behaviourally-based intervention with a strong emphasis on PA and nutrition. Study I developed and validated predictive equations for thigh composition assessment, using computed tomography (CT) as the reference method and with dual-energy X-ray absorptiometry (DXA) and anthropometry indicators as prediction models. Study II aimed the cross-sectional analysis of the associations between CRF and daily PA with thigh composition volumes determined by CT. In Study III a longitudinal approach allowed the investigation of the effects of a weight-loss intervention on thigh composition, CRF, and daily PA. Key results show that: a) prediction equations for the different thigh components may be useful in different settings where body composition may be critical and need to be followed longitudinally, such as in wasting illnesses; b) women with higher levels of CRF presented greater thigh skeletal muscle mass, independently from their PA level; c) greater low intensity PA was associated with lower thigh subcutaneous adipose tissue; d) maximal oxygen consumption level is associated with different thigh composition phenotypes; e) a 16-month weight-loss intervention positively impacted thigh low density SM tissue area, along with a decrease in low density SM quality and increases in CRF, and daily PA, suggesting the occurrence of the athlete's paradox in overweight and obese premenopausal women; f) beneficial changes were observed from 0-16 months in body composition determined by CT, DXA and anthropometry indicators simultaneously with improvements in CRF and in habitual PA.

Keywords: Obesity, body composition, computed tomography, exercise, cardiorespiratory fitness.

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Resumo Geral

O objectivo da presente Tese foi analisar a composição da coxa através das áreas de investigação dos métodos e das alterações da composição corporal, estudando, também, as suas associações com a aptidão cardiorrespiratória (ACR) e com a actividade física (AF), em mulheres pré-menopáusicas com excesso de peso e obesas. Foram realizados três estudos no âmbito do programa PESO (Promoção do Exercício e Saúde na Obesidade) - uma intervenção comportamental com grande ênfase na AF e na nutrição. No Estudo I procedeu-se ao desenvolvimento e validação de equações preditivas para a estimação da composição da coxa, utilizando como método de referência a tomografia axial computorizada (TAC), enquanto a densitometria radiológica de dupla energia (DXA) e a antropometria foram utilizadas como modelos preditivos. O Estudo II teve como objectivo investigar transversalmente as associações entre a ACR e a AF diária com os volumes da composição da coxa determinados pela TAC. O Estudo III recorreu a uma abordagem longitudinal para investigar os efeitos de uma intervenção de perda de peso na composição da coxa, ACR e AF diária. Os principais resultados demonstraram que: a) as equações preditivas para as diversas componentes da coxa podem ser úteis em diferentes contextos, nos quais a composição corporal possa ser crítica e necessite de ser monitorizada longitudinalmente; b) mulheres com níveis de ACR mais elevados apresentam maior massa muscular na coxa, independentemente do seu nível de AF; c) maiores níveis de prática de AF de intensidade leve estão associados a uma menor acumulação de tecido adiposo subcutâneo na coxa; d) o nível de utilização máxima de oxigénio encontra-se associado a diferentes fenótipos da composição da coxa; e) uma intervenção de perda de pesa com a duração de 16 meses promoveu o aumento da área de tecido muscular de baixa densidade, simultaneamente com a redução da qualidade desse mesmo tecido, bem como incrementos da ACR e da AF diária, sugerindo a

ocorrência do paradoxo do atleta em mulheres pré-menopáusicas com excesso de peso e obesas; f) dos 0-16 meses verificaram-se modificações benéficas na composição corporal determinada pela TAC, DXA e antropometria, simultaneamente com melhorias na ACR e na AF habitual.

Palavras-chave: Obesidade, composição corporal, tomografia axial computorizada, exercício, aptidão cardiorrespiratória.

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Abbreviations List

AT – Adipose tissue

BCa - Bias corrected and accelerated

BMI – Body mass index

CRF – Cardiorespiratory fitness

CT – Computed tomography

$DistalT_{Circ}$ – Distal thigh circumference

DXA – Dual-energy X-ray absorptiometry

EMCL – Extramyocellular lipid content

FFM – Fat-free mass (DXA)

FM – Fat mass (DXA)

FM_{Ll} – Lower limbs FM (DXA)

HD – Haemodyalisis

Hip_{Circ} – Hip circumference

HRR – Heart rate reserve

HU – Hounsfield units

ICC – Intraclass coefficient of correlation

IMCL – Intramyocellular lipid content

IPAQ - International Physical Activity Questionnaire

LST – Total body lean soft tissue mass (DXA)

LST_{Ll} – Lower limbs lean soft tissue (DXA)

MET – Metabolic equivalent

MRI – Magnetic resonance imaging

PA – Physical activity

PrT_{Circ} – Proximal thigh circumference

SD – Standard deviation

SEE – Standard error of the estimation

SM – Skeletal muscle

SMHU – Skeletal muscle quality quantified by Hounsfield units

SS – Sum of squares

T2DM – Type 2 diabetes mellitus

TEM – Technical error of measurement

TIAT – Thigh intermuscular adipose tissue

TSAT – Thigh subcutaneous adipose tissue

TTAT – Total thigh adipose tissue

VO_{2max} – Maximum oxygen uptake

%FM – Total body relative fat mass (DXA)

CHAPTER 1

General Introduction

"The beginning of knowledge is the discovery of something we do not understand."

Frank Herbert

Introduction to the Thesis

With the worldwide increase of obesity epidemic and its associated comorbidities, the treatment of this population became imperative. The accurate assessment of body composition changes in association with other health indicators, namely physical fitness and physical activity (PA) levels, before and after weight loss interventions, will allow a better understanding of the impact of different approaches to obesity treatment. The general aim of the current Thesis was to address this important issue within the scope of weight loss interventions in overweight and obese women.

More precisely, this Thesis was developed within the context of P.E.S.O. program (Promotion of Exercise and Health in Obesity), at the Faculty of Human Movement, between 2002 and 2004. P.E.S.O was a community program, empirically-based on the cognitive-behavioural approach, targeted to promote healthier lifestyles through PA and nutrition, in overweight and obese premenopausal women.

The present Thesis is based on independent research articles submitted or in press, in peer-review scientific journals with a recognized ISI Impact Factor. Despite the advantages, this format increases the risk of redundancy throughout the written document. So, to avoid repetition, the following structure was adopted:

- The general introduction is presented in *Chapter 1*, reviewing skeletal muscle (SM) and adipose tissue (AT) quantification by imaging methods state of the art, with special emphasis on thigh composition tissues, namely SM tissue and quality, as well as AT compartments, focusing methods to assess body composition at the tissue-organ level, such as computed tomography (CT) and magnetic resonance imaging (MRI). The associations between thigh components and a number of health conditions, including

obesity, were also reviewed, in addition to relations between thigh composition and, fitness and PA indicators, in obese subjects, before and after weight loss.

- In *Chapter 2* is offered a detailed description of the methods used in the current Thesis.

- *Chapters 3, 4, and 5* present the original research contributions. In *Chapter 3* it was addressed body composition methodology, through the development and validation of predictive equations for total thigh composition assessment, in pre-menopausal overweight and obese women, with dual-energy X-ray absorptiometry (DXA) and anthropometry-based prediction models using CT as the reference method. The associations, at baseline, between cardiorespiratory fitness (CRF) and daily PA, with thigh composition volumes determined by CT were analyzed in *Chapter 4*. In *Chapter 5*, a longitudinal design allowed the investigation of the effects of a weight-loss intervention on thigh composition, CRF, and daily PA in premenopausal overweight and obese women.

- Based on this original scientific research, *Chapter 6* discusses these findings within the range of the areas of body composition methods and alterations, and its interrelations with fitness and PA.

- Finally, in *Chapter 7* it is presented a summary of the main findings of this Thesis.

The Study of Body Composition

The living being's composition reflects net baseline accumulation of several substrates and nutrients acquired from the environment and retained by the body, empowering life. Components ranging from elements to tissues and organs make up the building blocks that give mass, shape, and function to all living things. Body composition analysis

techniques allow researchers to study how these building blocks function and change. The science of human body composition is organized into three interacting areas [1]: body composition rules and models (involves the components themselves, definitions and interrelations between them), body composition methodology (focuses on the available methods for *in vivo* and *in vitro* quantification of body components), and body composition variation (centers on the changes in body composition related to biological or pathological conditions), as showed in Figure 1.1.

Taking into consideration these three areas, the current Thesis, and particularly the research studies, are included in body composition methodology and body composition variation areas.

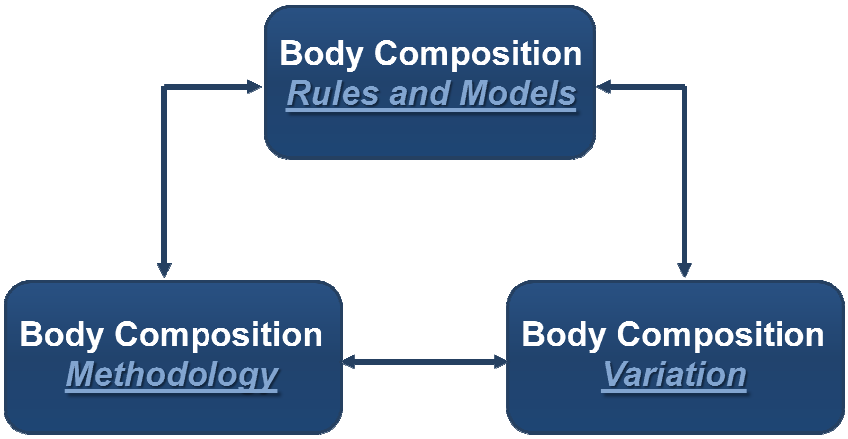

Figure 1.1. The study of human body composition. Adapted from Wang et al. (1992) [1].

The Five-Level Model of Body Composition

The central model in body composition research is the five-level model, in which body mass is considered as the sum of all components from five distinct and separate, but integrated, levels of increasing complexity: atomic, molecular, cellular, tissue-organ, and whole body [1], as presented in Figure 1.2. Considering this model, the thigh as the region of interest, and CT as the main body composition assessment technique in the

current Thesis, special scope will be focused at the tissue-organ level, particularly on SM tissue and AT.

Skeletal Muscle Tissue

The presence of muscle in the body occurs in three distinct forms: skeletal, smooth, and cardiac. Whole body SM mass is influenced by several modifying biological factors such as gender, age, ethnicity, physical activity, and disease [2].

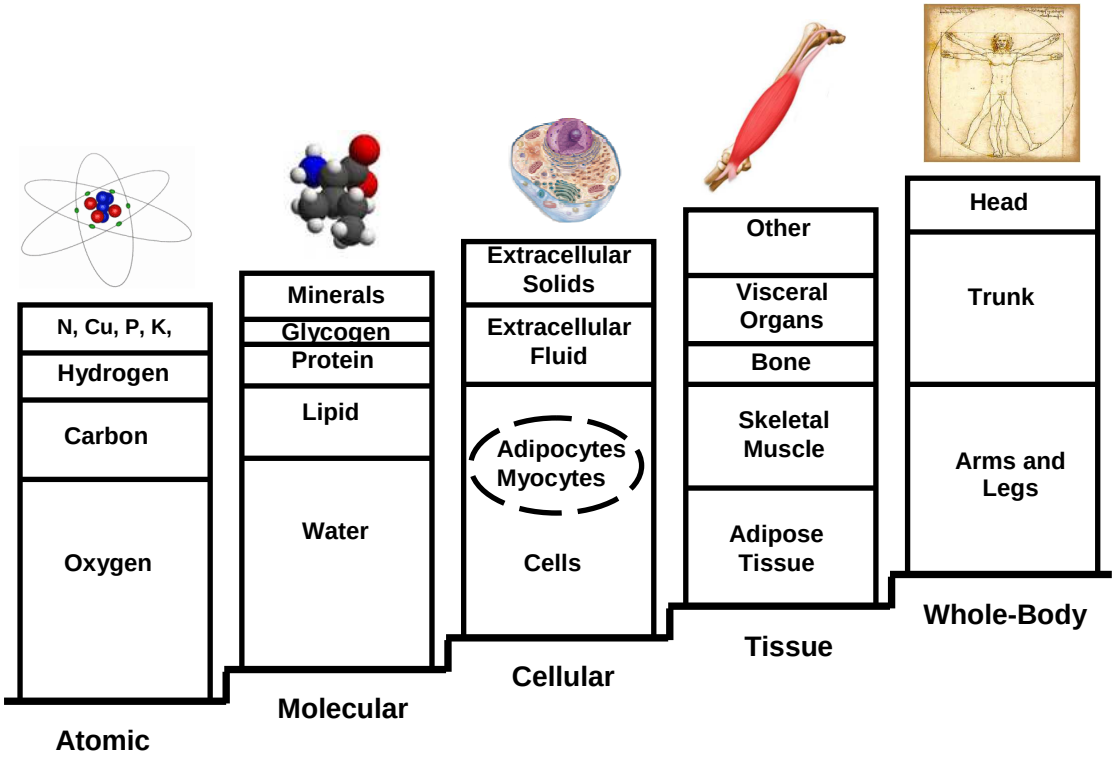

Figure 1.2. The five levels of human body composition. Adapted from Wang et al (1992) [1].

In adult humans, SM, also known as striated or voluntary muscle, is the largest component of body composition at the tissue-organ level, representing approximately 30% to 40% of the body mass of a healthy 58-kg woman or a 70-kg man, as showed by cadaver dissection [3].

The Visible Human Project of the National Library of Medicine, specifically the visible woman study, allowed the quantification of body composition based on MRI segmented images of the major body tissues of a woman cadaver [4]. As a result of the tissue-organ analysis, it was observed that SM was the second major contributor of whole body volume (25.6%), as well as in the upper and lower limbs (39.2% and 37.9%, respectively). In the trunk region the second greatest component was remainder (27.3%), while SM represented the third component (21.4%). A graphic illustration of the relations between total body SM and regional SM distribution is presented in Figure 1.3.

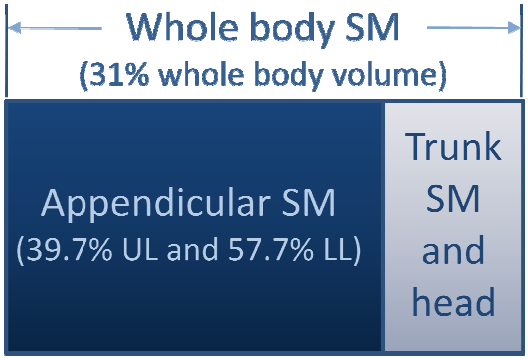

Figure 1.3. Relations between total body SM and regional SM distribution. Adapted from Kim et al. (2002) [5], and from Janssen et al. (2000). UL, upper limbs; LL, lower limbs.

The cross-sectional analysis of whole body MRI [6], revealed that SM mass relative to body weight was 38% in men and 31% in women. The same authors observed that, in what concerns SM distribution, women showed lower upper body SM, either in absolute and relative terms when compared to men (8.4 kg *versus* 14.1 kg, and 39.7% *versus* 42.9%, respectively), while in the lower body women presented lower absolute SM mass (12.2 *versus* 18.1 kg) but higher relative SM (57.7% *versus* 54.9%). These results indicate that women have a larger proportion of their total SM in their lower extremities in comparison to men.

The discrepancies between women SM results in Janssen et al. [6] and Shen et al. [4] studies may be caused by the different body composition assessment protocols used by

the researchers, and also by the sample size, because the data on the visible woman represent only one subject, and as consequence a bias enlargement may occurred.

Traditionally, CT has been used to quantify areas of SM, either by planimetry or by measuring the muscle area within a specific range of attenuation values (-29 to +150 HU – Hounsfield units)[7]. With this type of procedure, CT has been able to estimate the SM component of a region of interest excluding the visible AT, in and around muscle, providing more precise measurements [8]. In addition, the analysis of the attenuation characteristics of SM has provided information on the quality of the SM: a reduced muscle attenuation was interpreted to reflect an increased lipid component within muscle [9, 10]. This concept seems intuitive because as AT is negative on CT HU, an increase in muscle lipid content will reduce the attenuation of SM. The findings of a reduced muscle attenuation are consistent with an increased muscle lipid content determined histochemically [11, 12].

Accordingly to Mitsiopoulos et al. [7], considering tissue density, two types of SM can be distinguished, the first related to muscle tissue that includes interstitial AT, "anatomic SM", and the second specifically related to AT-free SM. Based on SM attenuation coefficients, several authors [13, 14] proposed a distinction between the two SM components: high density or normal density SM tissue (from +31 to +100 HU) and low density SM tissue (from 0 to +30 HU, representing lipid-rich SM, which includes fat components between and inside the muscle fibers). Other authors [7] proposed a different HU range for low density SM which includes HU from -29 to +30 HU.

Several changes occur in SM and its compartments, influenced by age and disease. Increasing age is associated with the loss of SM mass and function, and this occurrence is named sarcopenia (from the Greek words *sarx* - meaning flesh, and *penia* - meaning

loss). As a consequence of individual differences in peak SM mass and the rate at which SM decreases, SM mass varies widely in older adults [15].

Evidence from cross-sectional studies revealed that relative SM mass reduction seems to start in the third decade, while absolute SM mass seems to be fairly stable, on average, up to the end of the fourth decade, after which there are accelerating rates of loss in both genders - sarcopenia [6]. Longitudinal studies with CT measurements that have examined changes in SM mass or size obtained similar results, and allowed the estimation of a mean decrease in SM of approximately 6% per decade [16, 17].

As age progress, the rates of loss are greater for lower-body and thigh muscle than for upper-body and arm muscle [6]. The greater loss of SM in the lower body could be associated with the reduction in SM strength in the lower extremities [18, 19], and with the age related reduction in PA [20, 21], whom, as consequence, may contribute for the regional differences in sarcopenia. Accordingly to Janssen & Ross (2005) [15], it is reasonable to assume that a decline in PA would primarily be associated with a decreased use of lower body muscles, given that the muscles used for most common activities (i.e., walking, climbing stairs) are located in the leg and thigh. Considering the strong influence SM has on bone mineral density [22, 23], the increased prevalence of osteoporosis, particularly at the femoral neck, may be partially explained by the lower SM mass [6].

Besides the elderly, sarcopenia assumes particular interest in SM wasting diseases, such as haemodyalisis (HD) and HIV-infected patients. Subjects who underwent dyalisis treatment for chronic kidney disease exhibited more functionally significant SM wasting, determined by CT, than chronic kidney disease patients who were in a previous disease stage without HD. Furthermore, the reduction in SM cross-sectional area was associated with significant reduction in functional capacity, estimated by objective

measures of physical performance [24]. Loss of SM mass and limitations in activity have been reported in HIV patients, but Scott and colleagues [25] found that although impaired ability to activate knee extensors was associated with weakness and decreased specific force, the reduced central activation was not related to CT SM cross-sectional area. The authors suggest the possibility of different mechanisms contributing to SM impairment rather than atrophy, which reinforces the need for further research with larger and more diverse population in order to understand the nature and implications of these findings. Its importance relies on the fact that SM atrophy may be prevented with targeted exercise and/or improvement of other factors such as medication and nutritional factors.

The exact cellular and molecular mechanisms for the age-related loss of protein are not yet fully elucidated, but they are probably highly complex and involve multiple mechanisms, such as: impairments in neuromuscular function, alterations concerning the systemic environment (such as, testosterone, growth hormone/insulin growth factor-I axis, thyroid hormones, catecholamines and cytokines), and alterations intrinsic to the SM (myogenic regulatory factors, notch signaling pathway, myostatin and intracellular calcium) [26].

Age-related changes also take place in the composition of SM, reflecting an enlargement in SM lipid infiltration with increasing age, revealed through the measurement of CT attenuation coefficients in the thigh region [27-30]. This was observed even when changes in physical function were not detected, suggesting that dynamic remodeling of soft tissues occurs even in healthy, ambulatory, weightstable elderly subjects [31]. Similar findings were verified regarding the absence of mediation effects of several muscle parameters (including SM density) between PA and mobility limitation [32]. The decrease in SM density seems to be implicated in the reduction of

functional ability, as proposed by Visser et al. (2005) [28], who found that a greater fat infiltration into SM contributed to an increased risk of mobility loss in older men and women.

A greater lipid infiltration of SM is also related with increasing obesity, and is more relevant in obese patients with type 2 diabetes mellitus (T2DM) [13, 33-35]. These changes in lean-tissue density are due to an increased volume of lean tissue with attenuation values below the normal range for SM (<+31 HU). Despite the increase in low density SM in obesity, the volume of normal density SM (>+30 HU) seems to be unaffected, and varies relatively little among those subjects [13, 14, 34]. This occurred regardless of pronounced differences in body composition and overall midthigh volume. Insulin-stimulated glucose disposal was positively correlated with SM attenuation, in caucasian men and women, ranging from lean to obese (body mass index -BMI 19.6-41.0 kg/m^2) [9], and with insulin resistance in obese non-diabetic asian-pacific subjects [36]. Independently from total body adiposity, the area of low density SM revealed negative associations with insulin sensitivity, both in men and women, even after controlling for age. Furthermore, the area of low density SM accounted for 30% of the variance in insulin sensitivity, while the area of normal density SM was not associated with this parameter [13, 34]. So, it seems that low and normal density SM might have different metabolic implications thus requiring further investigation in different age groups and health conditions.

Other authors found associations indicating that lower SM attenuation determined with CT represents increased lipid content within muscle [13], supporting the association between the triglyceride content of skeletal muscle and insulin resistance, independent of obesity [37]. Recently, a set of clinical experiments with T2DM and obese non-DM subjects, supports CT as an extremely reliable technique for the assessment of SM lipid

stores producing results in the soleus muscle that are moderately correlated with the use of magnetic resonance spectroscopy as the reference method [38]. However, further testing in other SM groups, e.g., thigh SM, is warranted.

In healthy individuals, bone and muscle develop in harmony with the change in weight, but this adaptive physiological mechanism may be impaired in some older individuals that combine SM loss with obesity, determining a combination of excess weight and reduced SM mass and/or strength, defined as sarcopenic obesity [39, 40]. Considering the age-related changes in body composition, obesity and low SM mass (or strength), it is possible that they could coexist in the same subject simply by chance, but it is reasonable to hypothesize that they may be pathophysiologically connected. The imbalance between obesity and SM impairment, either defined by low SM mass or poor muscle strength, is associated with important negative health outcomes in older individuals [40, 41]. Epidemiological studies suggest that this syndrome is related to accelerated functional decline and high risk of diseases and mortality [28, 42] and, that when obesity and muscle impairment co-exist they act synergistically on the risk of developing multiple health related outcomes [43, 44], making the identification of these patients an essential goal in clinical settings. As a consequence, early screening starting in the fourth decade of life might be advised in order to identify subjects at risk for sarcopenic obesity and allowing for preventive measures to be implemented, before old age is reached.

Adipose Tissue (AT)

AT is a heterogeneous compartment consisting of adipocytes, extracellular fluid, nerves, and blood vessels. These components are dispersed throughout the body with varying metabolic functions according to their anatomic locations [45-48].

Accordingly to Shen et al. (2003) [49], AT can be divided into several components: the two main measurable components are subcutaneous and internal AT. Subcutaneous AT is defined as the layer found between the dermis and the aponeuroses and fasciae of the muscles, including mammary AT. Internal AT can be divided into visceral and nonvisceral components, and among this last component some small AT regions are specially named. That is the case of intramuscular AT, considered as the AT within SM fascicles, and of perimuscular AT, which is the AT inside the muscle fascia. The perimuscular AT is composed by intermuscular (between muscles) and paraosseal (in the interface between muscle and bone) AT. These small depots are difficult to measure: the perimuscular AT depots are small and not always visible when images are collected by CT or MRI acquisition, while the *in vivo* intramuscular layer can only be inferred from the attenuation coefficients of SM tissue. An adaptation of the proposed classification of AT by Shen et al. (2003) [49] is presented in Table 1.1, and a CT image of an axial plane of both thighs is showed in Figure 1.4.

Table 1.1. Classification of adipose tissue (AT) focusing on nonvisceral AT.

Adipose tissue compartment	Definition
Total AT	Sum of AT, usually excluding bone marrow and AT in the head, hands, and feet.
Subcutaneous AT	The layer found between the dermis and the aponeuroses and fasciae of the muscles. Includes mammary AT.
Internal AT	Total AT minus subcutaneous AT.
Nonvisceral internal AT	Internal AT minus visceral AT.
Intramuscular AT	AT within a muscle (between fascicles).
Perimuscular AT	AT inside the muscle fascia (deep fascia), excluding intramuscular AT.
Intermuscular AT	AT between muscles.
Paraosseal AT	AT in the interface between muscle and bone (e.g., paravertebral).

Adapted from Shen et al. (2003) [49].

AT is distributed throughout the human body and the pattern of distribution is influenced by many factors, including sex, age, genotype, diet, PA level, health status, and others conditions [13, 50-52]. As observed by cadaver dissection in the *reference man* study [3], AT represents approximately 21%, about 15 kg of body weight of a healthy 70-kg man. According to these authors, in men about 11% of total body is composed by subcutaneous AT, 7.1% is considered as "other separable" AT, 1.4% as interstitial AT, and 2.1% as yellow marrow (included with skeleton) AT.

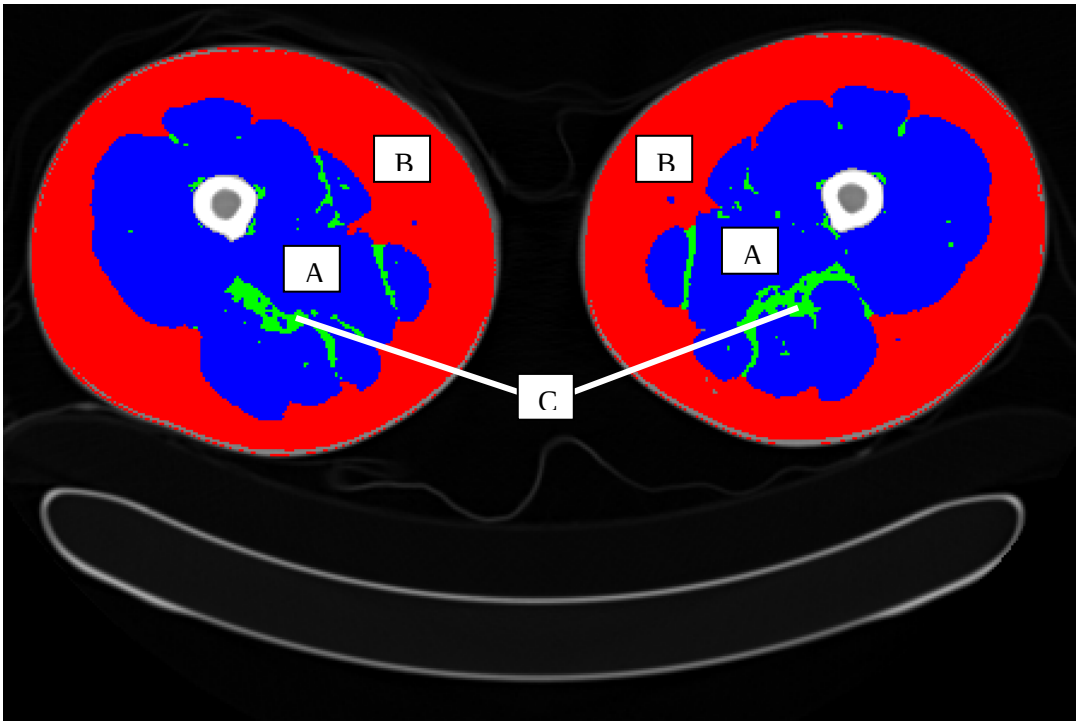

Figure 1.4. Axial plane of both thighs on a typical resolution CT scan. (A) Skeletal muscle tissue; (B) Subcutaneous adipose tissue; (C) Intermuscular adipose tissue.

The *visible woman* project [4], based on whole body MRI segmented images of a single female cadaver, revealed that subcutaneous AT presented the largest section of body volume (37.5%), followed by SM (25.6%), remainder (21.9%), head and neck (6.7%), visceral AT (5.2%), and lung (3.1%). Similarly, subcutaneous AT was the largest compartment in each region: 37.4% of trunk volume, 43.4% of upper limb volume, and 45.7% of lower limb volume. Remainder represented 27.3% of trunk volume, while

visceral AT and lung were the minor contributors of this body region volume (8.7% and 5.2%, respectively).

Sexual dimorphism was observed [53], with the use of whole-body MRI, indicating that total AT was significantly smaller in men when compared to women (18.8 ± 8.1 L *versus* 25.2 ± 11.9 L). Ethnic differences were found with MRI assessment, indicating that total AT was lower in African-american men than in Asian men, after controlling for age, height, weight and gender [54]. The same study revealed no ethnic differences for total AT in women.

Different health conditions may also influence total AT, as observed by Gallagher et al. [55], accordingly to which T2DM women presented less total AT than non-diabetic controls, while men with T2DM showed more total AT than controls. These results may indicate sex-specific differences in total adiposity in response to metabolic alterations.

Because total AT represents the sum of different AT depots, the implications of between-group differences in total adiposity should be considered in regard of the differences observed in its subdepots, reinforcing the relevance of measures of AT distribution in order to understand regional adiposity contribution.

Only in the last decades, AT distribution within the thigh, whether it's subcutaneous or located beneath the fascia, adjacent or inside SM, started to be investigated as ectopic depots of AT. The specific components that have been studied include subcutaneous AT (TSAT), intermuscular AT (TIAT), and intramuscular AT. This last component can only be inferred from SM density analysis, by means of the HU values, as previously referred, so it will be indicated as SM density or quality and it has already been addressed.

In what concerns total subcutaneous AT, cross-sectional data indicate that, although subcutaneous AT increases with age, subcutaneous AT as a proportion of total AT

decreases with advancing age, after approximately 35 years of age [53]. Differences between genders were observed with females presenting larger subcutaneous AT across the entire age range (23.7 L *versus* 16.5 L), representing approximately 94.0% and 87.8%, in women and men, respectively, indicating clear gender differences in AT partitioning. Similar results had already been observed with a single CT slice at midthigh, showing greater subcutaneous AT area in women than in men [13].

Accordingly to Shen et al. [53], in both genders, a smaller slope of the regression line between subcutaneous AT and age was found over the age of 50 years when compared with younger subjects, suggesting a decreased influence of the subcutaneous AT compartment in older ages [53]. More precisely, in women, subcutaneous AT was larger with increasing age before the age of 35 years and was smaller with increasing age after the age of 35 years, after adjustment for total AT and ethnicity.

When menopause status was taken into account, Shen et al. [53] observed an association between subcutaneous AT and age in premenopausal women, while in postmenopausal females no connection was found between the same variables. When pre and postmenopausal subjects were pooled together, menopausal effects were no longer significant, either as an individual term or as interactions with age. These results suggest that age and subcutaneous AT relationships should be considered when screening is performed in premenopausal women.

It is important to note that the sample used by Shen et al. [53] only included healthy subjects and the mean BMI was of 25.6±3.7 kg/m^2 and 25.5±5.4 kg/m^2 in adult males and females, respectively. Whether the inclusion of obese individuals in the study might have induced changes in the results, particularly if AT partitioning was considered as a function of total adiposity, is a matter that requires further research.

Yim et al. [54] found ethnic differences in subcutaneous AT, more precisely when Caucasians, African-americans and Asians were compared. Asian men and women revealed significantly less total subcutaneous AT than Caucasians and African-americans, after adjustment for age, gender, and total AT. Identical results were observed for femoral-gluteal subcutaneous AT [54].

Subcutaneous AT in the thigh represents the majority of total thigh AT area, comprising nearly 90%, either in lean, obese glucotolerant, and obese T2DM subjects [13]. Similarly to total subcutaneous AT, women showed greater subcutaneous femoral-gluteal AT than men [13].

Different health conditions may also be characterized by distinct subcutaneous AT distributions. Whole-body MRI revealed that subjects with T2DM presented significantly less total subcutaneous AT, including a lesser amount of femoral-gluteal subcutaneous AT, than control subjects [55]. Previous studies with obese subjects based on a single CT slice at midthigh reported greater areas of subcutaneous AT in obese glucose tolerant and obese type 2 diabetic subjects than in their lean counterparts [13].

A negative association was found between midthigh subcutaneous AT area and glucose tolerance indicators, in women, suggesting that increased thigh AT lipid content was associated with better glucose tolerance.

Accordingly to Kelley et al. (1991) [14], the analysis of midthigh CT scans in obese middle-aged males, revealed that increased AT and SM tissue contributed two-thirds and one-third, respectively, to increased thigh volume. Furthermore, the increment in SM verified in the obese was due to an increased volume of SM, with attenuation values below the normal range for SM, which enlarged in a reciprocal relationship with increasing obesity. Despite the increase in low density SM, the volume of normal density SM seemed to be unaffected by obesity [14].

In a multiethnic sample, femoral-gluteal subcutaneous AT was inversely associated with insulin and triglyceride concentrations, suggesting a negative relationship between these metabolic risk factors and subcutaneous AT located in the thigh region [54]. Previous reports by Snijder et al. [56] indicated that larger subcutaneous thigh AT was independently associated with more favorable blood glucose (in men) and lipid levels (in both genders), which is in agreement with the findings of Goodpaster et al. [57] who found that subcutaneous AT in the thighs of obese men and women was associated with a lower prevalence of the metabolic syndrome.

These results suggest that femoral-gluteal subcutaneous AT may have a protective effect on metabolic risk factors but the mechanisms thought to be involved in the protectiveness in relation to insulin resistance and cardiovascular disease are not fully clarified. Some authors suggest that femoral-gluteal AT may act as a reservoir for circulating non-esterified fatty acid [58]. Due to its lipolytic activity, with a relatively high lipoprotein lipase activity, subcutaneous thigh AT [59, 60] is more likely to take up non-esterified fatty acid from the circulation, protecting other organs against a higher exposure. Simultaneously, subcutaneous femoral-gluteal AT presents a low rate of fatty acid release [58, 61]. This way, ectopic fat storage is prevented, leading to a lower risk of insulin resistance [58, 62]. Another hypothesis is that there could be also regional differences in adipokines secretion, namely in subcutaneous thigh AT, that might contribute to the associations of this AT depot with glucose and lipid levels [56].

Regarding TIAT, several research studies quantified this component with the use of a single-CT slice measurement [13, 33], or by whole body MRI [63]. Comparisons of CT and MRI for TIAT data with corresponding cadaver analysis showed a high correlation (r=0.92) for leg and arm [7]. Gender comparison revealed that men showed significantly more TIAT than women (2.1±1.1 cm^2 *versus* 1.5± 0.9 cm^2) [64].

Accordingly to Goodpaster and colleagues [13], TIAT represented approximately 1.1% (1 cm^2) of thigh AT in lean subjects, 3.1% (5 cm^2) in obese glucose tolerant individuals, and 5.7% (9 cm^2) in obese diabetic patients, showing an enlargement in TIAT with increasing BMI and in T2DM. In what concerns obese subjects, similar results were obtained by the same authors in another study [33] showing TIAT absolute and relative contributions of 4.6 cm^2 and 3.0%, respectively.

Ethnic differences were observed in this thigh component revealing that with increasing adiposity, African American women and men accumulate greater deposits of TIAT than do whites or Asian females and males, and the difference grow across the range of total AT [63]. Asians showed TIAT levels similar to those of whites, but because they present higher amounts of total AT, they obtained, too, the highest proportion of total body AT as TIAT. So, an ethnic difference occurs for TIAT, and because of its location surrounding SM, it is possible that it will have implications in glucose regulation, suggesting that this type of AT distribution could interact with ethnicity in metabolic risk [63].

Concerning health implications of TIAT, it was showed that this distribution of AT deposition estimated by CT was an important body composition determinant of insulin resistance in obesity and T2DM. Specifically, TIAT was highest in obese DM, and although it accounted for only <3% of thigh AT, it was a strong marker of insulin sensitivity [13]. These results were confirmed by those of Larson-Meyer et al. [38], who, in addition, found that a 1-kg increase in body fat mass (FM), either in T2DM or in obesity, was associated with a 0.5-1.0 cm^2 increment in TIAT.

Findings obtained through MRI assessment performed in the whole body corroborated CT data, indicating that more TIAT was found in T2DM than in healthy non-T2DM control subjects [55], and that calf TIAT from subjects at increased risk for T2DM seem

to be involved in the pathogenesis of insulin resistance, as shown by the significant negative correlation with the glucose infusion rate in both women and men (r=-0.43 vs. r=-0.40, respectively) [64]. Yim et al. [65] obtained strong independent associations of TIAT, derived by MRI, with fasting glucose (P<0.001) and protein bound glucose (P<0.001), suggesting that TIAT may be related to glucose metabolism in Caucasian and African American healthy individuals; however, TIAT was also associated with total cholesterol in Caucasian. Furthermore, another research of the same group [45] revealed that although femoral-gluteal TIAT distribution varies by sex and ethnicity (Asian and African-American men had greater femoral-gluteal TIAT than Caucasians, adjusted for age and TAT), it presented an independent positive relationship with cardiovascular disease risk factors.

Some authors consider that AT interspersed around SM my impair muscle blood flow, reduce insulin diffusion capacity, or increase local concentrations of fatty acids, all of which have been shown to be associated with insulin-resistant glucose metabolism in SM [66-68]. Further, it has been suggested that an increase in TIAT contributes to insulin resistance through enhanced rates of lipolysis within SM [69]. Accordingly to other researchers, one possible explanation about the probable mechanism of action linking TIAT with insulin signaling is through triacylglycerol metabolites interfering with insulin signaling transduction, thereby altering whole body glucose and lipid metabolism [63]. These findings reveal that, although an association exists between TIAT and insulin resistance, controversy on the possible biological causes of these association remain, indicating the need of future research.

In what concerns thigh AT location and its metabolic implications, further research is warranted, particularly with longitudinal approaches, either in different ethnic groups, as well as in several health conditions, such as obesity.

Body Composition Assessment

The demands of the scientific community to estimate AT as a useful predictor of risk development of chronic diseases (such as ischemic heart disease, and non-insulin-dependent diabetes) has contributed to the increase of methods to measure this body tissue. Conversely, until recently, the assessment of SM tissue has been limited, but the increased awareness of the significance of developing and maintaining SM mass has stimulated the focus on approaches for the *in vivo* estimation of this metabolically active body component, reflecting multidisciplinary interests. Nevertheless, SM mass remain a difficult or impractical body component to accurately quantify in living humans.

The most accurate techniques to assess *in vivo* SM and AT are the two imaging methods: CT and MRI [70, 71], allowing the identification of tissue-organ components. MRI will not be addressed in detail in this review due to the fact that is not part of the methods used in the present Thesis. Inversely, because the molecular and the whole body levels will be analyzed through dual-energy X-ray absorptiometry and anthropometry, respectively, these methods characteristics will be referred in the current literature review.

Computed Tomography (CT)

Originally, CT was used in body composition research to estimate tissue dimensions, but this technique has been refined to allow the analysis of tissue composition [9, 34, 70, 72]. CT uses ionizing radiation and differences in tissue X-ray attenuation characteristics to construct cross-sectional images of the body [70]. The X-ray attenuation is expressed as the linear attenuation coefficient or CT number, which is a function of tissue density and chemical composition characteristics [73]. The CT

number is expressed in Hounsfield units (HU), on the basis of a linear scale using air and water as the reference (-1000 and 0 HU, respectively).

Cross-sectional CT images are composed of picture elements or pixels, usually 1mm by 1mm square, each of which has a CT number or HU value on a gray scale, that reflects the composition of the tissue. The lower the density of the tissue, the lower will be the HU values for the pixels that make up that tissue [34, 70, 73]. For example, AT density is lower than that of water, and the CT number for AT pixels ranges from -190 to -30 HU, while for SM ranges from -29 to +150 HU, and for bone tissue from +152 to +1000 HU [7, 74, 75]. These characteristics allow the determination of the tissue area (cm^2) for the different tissues in each cross-sectional image, using a computer-automated procedure that identifies the area of the target tissue, by selecting pixels within a given HU range [74].

Through multiplying the number of pixels for a given tissue by the surface area of the individual pixels, the area (cm^2) of the tissue is obtained. If multiple CT images are acquired, tissue volumes (cm^3) can be calculated by integrating cross-sectional area data from consecutive slices. As tissue densities for AT, SM and organs are fairly constant from one person to another, CT volume measures for these tissues can be converted to mass units by means of multiplying the volume by the assumed density values for that tissue [70]. For example, the assumed constant densities for AT and SM are 0.92 g/cm^3 and 1.04 g/cm^3, respectively [76].

Additionally, the average HU for SM can be used as an index of SM lipid content: the lower the average SM HU value (i.e., mean attenuation value), or the greater the number of low density SM pixels (e.g., <+30 HU), the higher the SM lipid content. But, it is important to note that, SM attenuation values by CT are not analogous to intramyocellular lipid values obtained by SM biopsy or proton magnetic resonance

spectroscopy, because CT is not capable of distinguishing between these two layers. Meaning that, SM attenuation determined by CT is a reflection of both intramyocellular and extramyocellular lipid content [70].

The validity and accuracy of area and volume measurements from CT have been established by comparing CT measures in human cadavers with direct measures (e.g., dissection, planimetry). A good agreement was found between CT and cadaver dissection for AT areas (correlations ranged from 0.77 to 0.94) [77], SM cross-sectional areas from the proximal thigh (correlations >0.95) [78], and SM and subcutaneous AT areas in the arms and legs (correlations from 0.97 to 0.99) [7].

For tissue quality, no comparisons have been made between CT measures of tissue composition and direct chemical extraction from human cadavers. So, the validity of CT has been determined by comparing the imaging methods with tissue biopsy samples in humans. CT-determined SM attenuation characteristics were well correlated with SM lipid levels determined in muscle biopsy samples, more precisely, between SM attenuation in the midthigh with SM triglyceride content and oil red O staining of lipid in muscle fibers, measured in percutaneous biopsy (correlations of 0.53 and 0.43, respectively) [11, 12, 34]. These results indicate that *in vivo* determination of SM by CT is associated with muscle lipid content.

In what concerns the reproducibility of tissue quantity by CT, in general, CT tissue area and volume are highly reproducible, revealing low coefficient of variation for repeated CT SM measurements in the thigh (1.4%) [79], and for whole-body AT volume (0.6%) [74]. Very little is known regarding the reproducibility of the measurement of tissue quality by CT. Goodpaster et al. (2000) [34] reported that the test-retest coefficient of variation for SM density was 0.51% in the thigh and 0.85% in the calf.

CT body composition assessment is being used in multiple-component analysis to quantify total body and regional AT, SM, bone, liver, and other organ-tissue volumes, across age groups, and disease states. By this means, is allowing to study the effect of aging, gender, ethnicity, obesity, weight loss, exercise, inactivity, type 2 SM size and quality, on associated function and metabolic implications [9, 13, 28, 34, 36, 45, 56, 57, 80]. The majority of these studies have based their observations on a single CT image, tipically, in the case of the SM, at the midthigh level [70].

Although CT is more accessible than MRI, exposure to radiation limits its use for multiple-image whole-body tissue quantification, and limits applicability in children and premenopausal women [81]. Thus, it is common for researchers and clinicians to use a single image (cross-sectional area, in cm^2) for the purpose of estimating whole body components. Some authors investigated how well the measurement of a single image could provide estimates of total body SM, AT and intermuscular AT, in healthy adults, whether its location was in the midthigh or in the abdomen (L_4-L_5), but due to ionizing radiation exposure in CT, these studies were conducted only with MRI [82-84]. Lee et al. (2004) [82], observed that thigh measures of SM resulting either from a single image, or from a succession of 7 consecutive images, obtained by MRI, were better predictors of whole-body SM, with smaller standard errors of the estimate (SEE), than abdominal SM measures. Nevertheless, SM in the abdomen was also a strong marker of whole-body SM which can be useful every time that a routinely abdominal single image is acquired to estimate FM in the abdomen. Shen et al. (2004) [83], obtained high correlations between SM and AT areas from a single abdominal slice and the corresponding total body SM and AT volumes, estimated by MRI. More precisely, these authors verified that the highest correlation with whole body SM volume occurred with SM area estimated 5 cm above L_4-L_5 level, while the best correlation for total body AT

volume was found with AT area 5 cm below L_4-L_5, with a relative small influence of age, sex, ethnicity and prone or supine imaging collection position. Other authors (Ruan et al., 2007)[84] estimated whole body IMAT volume from single-slice IMAT areas at different body locations, assessed by MRI, in a multiethnic population. Accordingly to these authors, midthigh was the near best single predictor in all ethnic groups, with adjusted R^2 ranging from 0.49 to 0.84. When a second and third slice were added, an increase in R^2 occurred (ranging from 0.49 to 0.92), along with a reduction of the standard error of the estimate, which ranged from 0.15 L to 0.36 L, independently from menopausal status and degree of obesity. So, although individual applicability of a single-slice image is limited, the literature indicates that group studies could be conducted economically using a single cross-sectional SM and AT areas.

Merely a few studies have made a direct comparison between CT and MRI. Engstrom and colleagues [78] compared SM cross-sectional area measurements in the midthigh of three cadavers to corresponding MRI images, and found that CT and MRI measures were highly correlated for each muscle (r=0.81 to 1.00). Two other studies, that combined a total of 18 subjects, compared CT and MRI measures of subcutaneous AT and found correlation coefficients ranging from 0.98 to 0.99 [85, 86]. Mitsiopoulos et al. [7] reported a strong correlation between CT and MRI measures for SM (r=0.97) and subcutaneous AT (r=0.99), obtained from an arm and leg from each of two cadavers.

These findings indicate that additional research is required to compare CT and MRI images in larger and more heterogeneous samples regarding age, gender, ethnicity, BMI status, among others, in order to determine the degree of interchangeability of these methods in the evaluation of the different tissue-organ components of the body. Only after the agreement between the two techniques is established it will be possible to

accept MRI findings (such as those regarding single slice *versus* multi slice results) to be used in CT assessments, and *vice-versa*.

Dual-energy X-ray Absorptiometry (DXA)

Another imaging method used to estimate body composition, not at the tissue-organ level but at the molecular level, is dual-energy x-ray absorptiometry (DXA). Originally, DXA was applied to assess bone mineral content and density, but technology has been refined for the analysis of soft tissue mass [87]. DXA assesses body composition by measuring the attenuation of X-rays emitted using pencil- or fan-beam technology at two energy levels as it traverses the body [88]. DXA software allows the distinction between bone mineral and soft tissue, after which occurs the soft tissue separation into fat and lean soft tissue (LST).

Beside these estimations, DXA software permit the definition of particular regions of interest, such as the thigh, providing specific information about bone mineral, fat and lean soft tissue. Although there is a good agreement between DXA regional body composition assessments and whole body scans, in the precision found for bone mineral density, fat, and lean soft tissue mass, regional measurements (lower and upper limbs, trunk, pelvis, and spine) seem to be less precise than total body measurements [89, 90].

Measures of total and regional fat-free soft tissue mass (coefficient of variation from 1 to 7%) [5, 91, 92] and FM (coefficient of variation from 1 to 7%) [91] using either pencil- or fan-beam technology are highly repeatable, but results may differ between the two methods or model types (e.g., Hologic, Lunar, etc.) [93]. Besides to the scanner type (pencil- or fan-beam), estimations of fat and lean soft tissue mass using DXA are affected by the software used (algorithms), the sagital diameter, and the hydration status of the subject [94]. Other limitations of DXA applicability regarding obesity studies are

due to the size of the subject, as obese individuals may exceed the weight (114 to 159 kg) and size (193–197 cm by 58–65 cm) limits of the equipment, depending on the manufacturer and model [94]. Additionally, increased tissue thickness, such as that found in obese individuals, is associated with a phenomenon called beam hardening that may induce an underestimation of the accurate fat content [91, 95].

DXA scanner is relatively easy to use in most populations as it requires very little effort from the participant [94]. Furthermore, DXA measurements involve less radiation exposure than CT, are less time consuming, and readily available at a significantly lower cost, contributing to the increasing use of this technique at different settings.

To our knowledge, only a few studies in obese humans have examined the ability of DXA to predict SM as compared to criterion methods, such as the four-compartment model or CT and whole-body MRI. DXA estimates of appendicular FM are strongly associated with CT AT mass (r = from 0.91 to 0.99) [95, 96]. Equally, DXA measures of total or appendicular lean soft tissue mass are highly associated with matching values obtained by CT or MRI (coefficient of correlation from 0.86 to 0.98) [5, 92, 95-97], although in one study DXA minimally overestimated SM in healthy (2.0 kg and 5.8%) and HIV-infected men (1.4 kg and 5.1%) [98]. Similarly, a close association has been observed between total FM, as estimated by a four-compartment model, and fan-beam DXA (r = 0.98) [99]. Recently, Bredella et al. (2010) [100] reported that DXA underestimated thigh fat and overestimated thigh muscle mass when compared to CT, and this error increased with increasing weight, in premenopausal women, within the phenotypic spectrum ranging from obesity to anorexia nervosa. So, accordingly to these authors, DXA may not accurately assess body composition in markedly obese women. The same authors referred, also, that the level of hydration affected DXA estimates of thigh fat.

When DXA was used to evaluate changes in body composition in obese subjects, it was observed that DXA overestimates alterations in FM, and underestimates modifications in lean soft tissue mass, compared with criterion measures, such as the four-compartment model or CT: r=0.53 to 0.55 regarding lean soft tissue changes, and r=0.66 to 0.90 concerning FM modifications [101-104]. Concerning the thigh region, in relatively healthy older men, DXA overestimated thigh SM before and after a strength training intervention. Additionally, DXA was not able to detect small changes in this body composition component resulting from a strength training intervention, because the error associated with DXA was greater than the SM change, which did not occur with CT results [105, 106].

So, although, in comparison with criterion techniques, DXA has the ability to detect FM and lean soft tissue changes in subjects that lost body weight, improvements to DXA are needed to achieve accurate assessments of small changes in thigh SM mass, in order to validate DXA as a reliable measure in that context [107].

The discrepancies observed between DXA and the two imaging reference methods may be partially caused by the different components estimated by each technique: DXA provides the proportion of fat in each pixel, estimating measures of fat rather than AT, unlike CT and MRI. Furthermore, CT differs from DXA in that pixels are allocated to AT or to lean tissue depending on levels of HU, and are not graded as mixtures of lean tissue and AT [88]. Nevertheless, the strong correlation found between DXA and CT measures of SM and AT (as reported previously), indicate that DXA provides a cheaper, faster, and more readily available alternative to CT and MRI, with lower radiation exposure than CT.

Anthropometry

Anthropometry represents a whole-body approach for body composition estimation, that includes measures such as weight, stature, circumferences, skinfolds, and body diameters. In the current literature review, only circumference measures will be addressed. The instruments needed are portable and relatively inexpensive, comprising noninvasive procedures. Training is required by an experienced professional and quality control, including analyses of reliability data and calibration of the equipment, should be performed during a study, and during the course of clinical work [108].

The interpretation of an anthropometric value is based on the assumption that the tissues included in the measurement are in a *standard* state, for example, that muscles are fully relaxed, and soft tissues are normally hydrated. If these conditions are not full field, data interpretation may be invalid. Almost all anthropometric variables include a variety of tissues, and their separate influences on recorded values are not always clear [108]. For example, the enlargement of SM or subcutaneous AT increases girth measurements, and standing for 1 to 2 hours, or prolonged sitting, causes an accumulation of extracellular fluid in the lower limbs, leading to increases in ankle and calf circumferences [108].

Another assumption, when anthropometry is used in body composition, referrers that tissue composition is independent of tissue size, but this assumption may be violated [108]. For instance, the relative fat content of AT presents a direct association with subcutaneous AT thickness within age groups [109-111], and the fat content increases with the enlargement of subcutaneous AT during growth and aging [112, 113].

From the several anthropometric techniques available, skinfold thickness have been extensively used to assess subcutaneous AT, in order to estimate total body density, as proposed by Jackson & Pollock, or Durnin & Womersley, for men and women, of varying age and body composition [114-116], and to correct girth results in the

estimation of SM mass, as developed by Martin et al. [117], or Doupe et al. [118] in elderly cadavers, or by Lee et al. [119] in adults of both genders and different weight conditions (non-obese and obese). Nevertheless, there is some concern about the accuracy of skinfold measurements in obese populations [120, 121], namely, anatomic points might be difficult to identify, the entire depth of the skinfold is hard to grab, and larger amounts of subcutaneous AT turn into a difficult task the elevation of the skinfold with parallel sides, increasing intra- and inter-observer errors. Also, the maximum jaw opening of the calipers might make it impossible to measure skinfold thickness.

Studies including obese individuals should consider using alternative anthropometric techniques, such as circumferences. The analysis of the associations between multiple skinfold thicknesses and girths revealed that these measures were highly correlated, and that circumferences were fairly more reliable method than skinfold thickness at the same sites, in overweight subjects. In addition, the low correlations between these two techniques indicate that the sets of skinfold and girth measures are not interchangeable [122-124].

Accordingly with Bellisari & Roche [108], in terms of body composition, limb circumferences are difficult to interpret, because they include skin, subcutaneous AT, muscle, bone, blood vessels, nerves, and deep AT. The interpretation of hip circumference is uncertain since it includes large amounts of AT and SM, and it is also affected by pelvic size and shape. Limb circumferences corrected for skinfold thicknesses can be used to estimate muscular development. Circumferences are assessed with a tape while minimal tension is applied so that the soft tissues will not be compressed; as a consequence, an enlargement of subcutaneous AT or SM caused by edema will increase the recorded measurements [108].

In what regards limb composition, anthropometry alone does not provide the most accurate predictions of AT and SM mass. In fact, some assumptions that have been used in anthropometric indexes of limb composition have proven to be incorrect, such as those concerning: cross-sections of the limbs are circular; a skinfold thickness is equal to the thickness of subcutaneous AT at the site; subcutaneous AT in a cross-section of a limb is of constant thickness [108]. When matched up to MRI, a general level of fatness is measurable by anthropometry and MRI over a range of subjects. However, the pattern of fat thicknesses assessed over a number of specific sites by one method of measurement is unlikely to be duplicated by the other method on the same individual [125]. When compared with CT or MRI data, the anthropometric approach overestimates SM plus bone cross-sectional areas in the limbs, despite SM plus bone areas from CT or MRI include AT and connective tissue within SM. In the elderly and the obese, this overestimation is larger [126, 127], partly due to increases in intra- and intermuscular AT, which are not related to the amounts of subcutaneous AT [128, 129]. The functional capacity of SM can vary independently of its volume, as occurs when its water content increases in severe protein-caloric malnutrition [130], and when an enlargement in intra- and intermuscular AT take place, as in obesity and in the elderly.

A literature review focused on thigh SM and AT predictive equations, based on anthropometry and using imaging methods as the reference, is presented in Tables 1.2 and 1.3. The first thigh SM and AT equations are currently used and their development was based in mathematical functions derived from the anthropometric assumptions already mentioned. All the other equations were anthropometry based, with the exception of Elia et al. equation [131] that estimates SM mass also from DXA assessment. In what concerns the reference method used, only Overend et al. [132] used CT as the reference for several SM area and volume estimates, while the other equations

Table 1.2. Thigh SM tissue prediction equations.

Reference	Sample	Method	Reference Method	Predicted Variable	Equation	SEE/R^2
				Midtghigh area (cm^2)	MTA = MTC^2 / 4π	
				Midthigh SM plus bone area (cm^2)	SMBA = (MTC - π x TSF/2)^2 x 4π	
				Limb SM CSA (cm^2)	TMA = (MTC^2 / 4π) – (MTC x TSF / 2) - BA	
				Thigh SM CSA (cm^2)	TMA = (MTC^2 / 4π) – (MTC x TSF / 2) - 6	
Wright & Heymsfield (1984)				Thigh SM area (cm^2)	TMA = (MTC – π x TSF)^2 / 4π	
Overend et al. (1993) [132]	N=24 (M) *Young men* n=13 Age=24.5±5.4 y Weight=70±10.7 kg *Old men* n=11 Age=71.0±4.6 y Weight=75.0±6.9 kg	Anthropometry	Thigh CT		*Young men*	
				Total quadriceps CSA (cm^2)	TtQA = (MB CSA x 0.31) + 28.0	R^2=0.86 SEE=5.4 cm^2
				Total quadriceps volume (cm^3)	TtQV = (MB Vol x 0.45) – 51.3	R^2=0.96 SEE=58.7 cm^3
				Quadriceps SM CSA (cm^2)	QSMA = (MB CSA x 0.30) + 27.6	R^2=0.86 SEE=5.2 cm^2
				Quadriceps SM volume (cm^3)	QSMV = (MB Vol x 0.43) – 58.4	R^2=0.96 SEE=56.5 cm^3
				Total hamstrings CSA (cm^2)	TtHA = (MB CSA x 0.55) – (TtT CSA x 0.35) + 14.7	R^2=0.62 SEE=4.4 cm^2
				Total hamstrings volume (cm^3)	TtHV = (MB Vol x 0.17) + 39.0	R^2=0.47 SEE=109.6 cm^3
				Hamstrings SM CSA (cm^2)	HSMA = (MB CSA x 0.53) – (TtT CSA x 0.35) + 14.7	R^2=0.61 SEE=4.2 cm^2
				Hamstrings SM volume (cm^3)	HSMV = (MB Vol x 0.15) + 53.0	R^2=0.43 SEE=107.6 cm^3

Table 1.2. Thigh SM tissue prediction equations *(continuing)*.

Overend et al. (1993) [132]				*Old men*		
				Total quadriceps CSA (cm^2)	TtQA = (MB CSA x 0.52) – 17.4	R^2=0.82 SEE=3.8 cm^2
				Total quadriceps volume (cm^3)	TtQV = (MB Vol x 0.39) + 12.4	R^2=0.84 SEE=83.3 cm^3
				Quadriceps SM CSA (cm^2)	QSMA = (MB CSA x 0.52) – 22.4	R^2=0.81 SEE=3.8 cm^2
				Quadriceps SM volume (cm^3)	QSMV = (MB Vol x 0.38) – 94.4	R^2=0.80 SEE=93.0 cm^3
				Total hamstrings CSA (cm^2)	TtHA = (MB CSA x 0.29) – 11.8	R^2=0.65 SEE=3.2 cm^2
				Total hamstrings volume (cm^3)	TtHV = (MB Vol x 0.13) + 196.2	R^2=0.47 SEE=62.7 cm^3
				Hamstrings SM CSA (cm^2)	HSMA = (MB CSA x 0.28) – 15.7	R^2=0.64 SEE=3.2 cm^2
				Hamstrings SM volume (cm^3) Total quadriceps CSA (cm^2)	HSMV = (MB Vol x 0.13) + 83.0	R^2=0.37 SEE=73.4 cm^3
Housh et al. (1995) [133]	N=43 (M) Age=25±5 y Weight=81.1±12.8 kg	Anthropometry	Midthigh MRI	Thigh SM CSA (cm^2)	TMA = (4.68 x MTC) – (2.09 x AntTSF) – 80.99	R=0.86 SEE=9.5 cm^2
				Quadriceps CSA (cm^2)	QA = (2.52 x MTC) – (1.25 x AntTSF) – 45.13	R=0.86 SEE=5.2 cm^2
				Hamstrings CSA (cm^2)	HA = (1.08 x MTC) – (0.64 x AntTSF) – 22.69	R=0.75 SEE=3.5 cm^2

Table 1.2. Thigh SM tissue prediction equations *(continuing)*.

Knapik et al. (1996) [134]	N=18 (9M; 9F) Age=23±5 y *Male* n=9 Age=21.0±2.3 y Weight=81.6±7.0 kg *Female* n=9 Age=25.2±5.5 y Weight=59.6±7.0 kg	Anthropometry	Midthigh MRI	Thigh SM CSA (cm^2)	TMA = 0.649 x $[(C_r/\pi - TSF)^2 - (0.3 \times d_E)^2]$	R=0.96 SEE=11.3 cm^2
Elia et al. (2000) [131]	N=16 (8M; 8F) Age=41-62 y *Male* n=8 Age=43-62 y BMI=28.6±5.4 kg/m^2 *Female* n=8 Age=41-60 y BMI=25.1±5.4 kg/m^2	Anthropometry and DXA	Thigh MRI	Limb SM mass (kg)	*DXA* SMM = LimbM - FM - 1.82 x BAsh *DXA and anthropometry* *Men* SMM = (1.029 x LimbM) – (1.286 x FM) – (2.800 x BAsh) – (0.905 x skin) *Women* SMM = (1.037 x LimbM) – (1.297 x FM) – (2.824 x BAsh) – (0.913 x skin)	Not indicated

AntTSF, anterior thigh skinfold (mm); BA, bone area (cm^2); BAsh, bone ash mass (kg); C_r, total thigh circumference; CSA, cross-sectional area; CT, computerized axial tomography; COPD, chronic obstructive pulmonary disease; CTG, thigh circumference corrected for the front thigh skinfold thickness (cm); d_E, distance across the medial and lateral femoral epicondyle (cm); DXA, dual-energy X-ray absorptiometry; F, female; FM, fat mass (kg); HA, hamstrings area (cm^2); HSMA, hamstrings skeletal muscle cross-sectional area (cm^2); HSMV, hamstrings volume (cm^3); LimbM, limb mass (kg); M, male; MB CSA, muscle plus bone cross-sectional area (cm^2); MB Vol, muscle plus bone volume (cm^3); MRI, magnetic resonance imaging; MTA, midthigh area (cm^2); MTC, midthigh circumference; QA, quadriceps area (cm^2); QSMA, quadriceps skeletal muscle cross-sectional area (cm^2); QSMV, quadriceps skeletal muscle volume (cm^3); SMBA, skeletal muscle plus bone area (cm^2); SMM, skeletal muscle mass (kg); TMA, thigh muscle area (cm^2); TMV, thigh muscle volume (cm^3); TSF, thigh skinfold thickness (mm); TtHA, total hamstrings cross-sectional area (cm^2); TtHV, total hamstrings volume (cm^3); TtQA, total quadriceps area (cm^2); TtQV, total quadriceps volume (cm^3); TtT CSA, total thigh cross-sectional area (cm^2).

Table 1.3. Thigh AT prediction equations.

Reference	Sample	Method	Reference Method	Predicted Variable	Equation	SEE/R^2
				Midtghigh area (cm^2)	MTA = MTC^2 / 4π	
				Midthigh subcutaneous AT area (cm^2)	MSAT = MTA - SMBA	
				Limb (thigh) subcutaneous AT area (cm^2)	TSATA = (MTC x TSF) / 2	
Tothill et al. (2002) [135]	*Group 1* N=10 (8 M; 2 F) Age=23-49 y *Male* n=8 Age=35.0±7.3 y BMI=24.3±1.4 kg/m^2 *Female* n=2 Age=30.3±7.8 y BMI=19.6±0.4 kg/m^2	Anthropometry	Thigh MRI	Anterior thigh subcutaneous AT thickness (mm)	TSATT = [(0.40 x TSF) / 2] + 0.51	R^2=0.17 SEE=2.2 mm

F, female; M, male; MRI, magnetic resonance imaging; MSAT, midthigh subcutaneous AT area (cm^2); MTA, midthigh area (cm^2); MTC, midthigh circumference (cm); SMBA, skeletal muscle plus bone area (cm^2); TSATA, thigh subcutaneous adipose tissue area (cm^2); TSATT, thigh subcutaneous adipose tissue thickness (mm); TSF, thigh skinfold thickness (mm).

were MRI based. The predicted variable of the equations involving MRI were thigh SM and several thigh SM groups (quadriceps and hamstrings) cross-sectional areas, and also subcutaneous AT thickness. One weakness of all the equations, except in what concerns the mathematical derived formulas, is that they were developed with small samples, thus limiting its validity and reliability. Other limitations concern the low R^2 obtained for hamstrings cross-sectional areas and volumes, in both young and old men [132], as well as for the anterior thigh subcutaneous AT thickness estimates [135], or even the absence of this analysis [131].

Regardless of the referred limitations, anthropometry is an essential component of body composition, and of body composition predictive equations. Furthermore, anthropometric assessment, and the body composition values to which they are related, are significantly associated to health, namely with obesity and related morbidity [136-139], and mortality [136, 140-142]. Nevertheless, studies that employ research-based imaging methods such as CT and MRI, that quantify the major body composition components at the tissue-organ level, and the extent to which they could be predicted from anthropometric measures, linking body composition and health status, deserves further development in future research, updating anthropometry applicability to the whole body and in several ectopic AT depots (such as liver, perivascular, kidney, pancreatic, and SM), as well as to other body composition components.

Based on the wide range of known body composition models and measurable properties, scientists, clinicians and trainers, can quantify several body components and, with longitudinal assessment, can track changes in health, disease and sports, with implications for understanding efficacy of nutritional, clinical and training interventions, as well as diagnosis, prevention, and treatment at different settings.

Body Composition *versus* Physical Activity and Physical Fitness

As described previously, the analyzed thigh components vary influenced by several factors such as gender, age, and health status. Other factors affecting SM and AT changes include PA and physical fitness.

PA has been widely recognized as an important health contributor, with a major role in the prevention and treatment of several non-communicable diseases, independently from gender, and across lifespan, representing a key determinant to energy expenditure, and thus fundamental to energy balance and weight control [143-145]. Accordingly, PA is recommended as a component of weight management for: prevention of weight gain, weight loss, and prevention of weight regain after weight loss [146]. In order to prevent weight gain, evidence supports the effectiveness of moderate intensity PA between 150-250 min/wk. Modest weight loss can be achieved with the same PA dose, as long as diet restriction is moderate, while clinically significant weight loss have been associated with greater amounts of PA (>250 min/wk). There seems to be a dose effect of PA, with greater weight loss and enhanced prevention of weight regained with doses of moderate intensity PA that approximate 250-300 min/wk (around 2000 kcal/wk). Resistance training does not appear to enhance weight loss but may increase SM mass and loss of AT mass, contributing also to decreases in quite a lot of chronic disease risk factors. Lifestyle approaches for increasing PA should be a strategy included in weight management efforts, although there is a need to better document this association [146].

A strong direct association between PA habits and physical fitness is accepted. One of the major components of health-related fitness is aerobic capacity or CRF, usually indexed as peak oxygen consumption (peak VO_2), fully recognized as an important health contributor. Epidemiologic studies indicate that individuals with low CRF are much more likely to develop hypertension [147, 148], diabetes [148-150], and

metabolic syndrome [151, 152], and to present higher rates of death due to cardiovascular disease [153, 154], cancer [155, 156], and all causes [153, 157, 158] during follow-up. Those results were observed independently from gender, age, and BMI. The relevance of these findings is enlarged by the high proportion of adults presenting a low functional aerobic capacity, specially along with obesity and sedentarism, which increases mortality risk [159]. A maximal oxygen consumption of no greater than 18 mL/kg/min has been identified as indicating disability and is used as a threshold value of independent living [160].

Accordingly to Fleg et al. (2005) [161] a decline in peak oxygen consumption per unit of time of 3% to 6% per decade occurs for the third and fourth decades of life, but after 70 years of age, the rate accelerated to more than 20% per decade. So, in both genders, the levels of CRF tend to decline nonlinearly at an accelerated rate after 45 years of age [161, 162]. This decrease seems to enlarge with the increment of BMI, as reported by Jackson et al. (2009) [162], who observed a reduction of 0.20 metabolic equivalents (METs) in CRF of women, for each unit of increase in the BMI. A similar trend was verified in men but the reduction was of 0.32 METs for each unit of BMI raise. In obese subjects, an association between decreased oxidative capacity and reduced SM quality was also found [163], indicating that SM of obese individuals seems to be poorly equipped for substrate oxidation, revealing a low maximal aerobic capacity [9].

Aerobic or/and resistance training have been used in intervention programs, mainly, among the elderly, in order to induce body composition changes and, particularly, modifications in thigh composition, with or without weight loss. Several studies analyzing the impact of aerobic PA on CRF and thigh composition without weight loss have been performed. In what regards thigh components and PA, a 13-week intervention of moderate intensity aerobic exercise without weight loss or caloric

restriction, revealed significant increases in normal density SM area and mean SM attenuation coefficients, determined by MRI, in lean and obese (glucose-tolerant and T2DM) middle-aged men. The results indicated substantial reductions in SM lipid content in both obesity and T2DM groups, along with CRF improvements, despite no change occurred in body weight [164]. Another intervention, however in a randomized controlled trial approach, revealed similar results concerning SM lipid content in older men and women enrolled in a one year combined exercise intervention (aerobic, resistance, flexibility, and balance exercise), when compared with the control group. These changes were depot specific, since subcutaneous AT was not significantly different in PA and control groups, indicating the contribution of PA to the prevention of the age-associated rise in SM lipid infiltration [164, 165]. Analogous outcomes were presented by middle-aged, overweight, HIV-infected women, in a randomized controlled trial, as a result of a 16-wk aerobic exercise intervention when compared to a control group. Besides increased midthigh SM attenuation coefficients and no changes in subcutaneous AT, these women revealed, also, an increment in SM area alongside with significant developments in CRF [166].

In what regards resistance exercise, recently, when a PA intervention with high intensity progressive resistance training was implemented in young and octogenarian women [167], CT-measured thigh SM area significantly increased in young but not in older women, suggesting that the ability to hypertrophy with a training program diminishes with advancing age. Previous studies with older women have showed different findings, indicating significant increases in thigh SM cross-sectional area and volume, as a result of resistance training interventions, with 3 sessions per week, lasting 9 to 16 weeks [168, 169]. Decreases in thigh subcutaneous AT were also reported [168]. Further studies are needed to clear this controversy, and to determine whether these effects on

SM and AT without weight loss occur also in younger women, with a special focus on overweight and obese conditions, as a means to prevent/retard sarcopenic obesity with increasing age.

When aerobic PA interventions were combined with caloric restriction in order to achieve weight loss, some studies refer greater weight loss reductions found in diet combined with aerobic exercise [51, 170, 171], while others revealed similar losses in both diet only, and diet combined with aerobic exercise in both groups [172]. Shorter intervention durations (16-week), combining aerobic or resistance exercise with energy restriction, showed preservation or significantly lower decreases of appendicular and thigh SM tissue, compared to greater losses in participants who were only dieting [51, 171-173]. In a longer intervention, no significant changes were reported in SM area in both diet and diet plus aerobic exercise groups. Similar reductions in low density SM tissue occurred in both groups, as well as decreases in subcutaneous AT, but with a greater magnitude among the participants that performed aerobic exercise [170]. These results occurred alongside with improvements in CRF of the exercising groups, and significant decreases of this physical fitness indicator in the groups who only underwent diet [170, 171]. Another intervention with 6-months duration [174], but involving aerobic exercise and aerobic exercise with weight loss groups, revealed similar increments in CRF in both, together with identical decreases in thigh subcutaneous AT area. In what concerned low density SM area, aerobic exercise alone did not altered this thigh component, but the addition of weight loss originated significant decrements in midthigh low density SM area, in previously sedentary older men. A greater weight reduction was also achieved in this group [174]. These evidences seem to indicate that, in weight loss interventions, the combination of energy restriction with aerobic exercise improves maximal functional capacity, and results in a greater weight and AT reduction,

preservation or lessening of SM loss, and a higher decrease of low density SM area. It is, also, important that these findings be validated in a larger population, with different age groups and health conditions.

Gaining and loosing weight are known to affect the different AT and SM compartments, at the whole body and regional levels. Changes in subcutaneous AT, intermuscular AT, SM, and HU need further research in order to be documented, preferably in a longitudinal basis.

Research Goals

The aims of the original research that oriented the present Thesis were:

- To develop and validate predictive equations for thigh composition based on readily available methods, namely DXA and anthropometry, using CT as the reference, in overweight and obese premenopausal women.
- To analyze the associations between CRF and daily PA with thigh composition variables determined by CT, in overweight and obese premenopausal women.
- To detect changes in thigh composition and whole body composition indicators from a 16-months weight loss program, in a cohort of overweight and obese premenopausal women.

These research goals combined provide a comprehensive analysis of body composition methods and alterations research areas, with focus on thigh composition, as important investigation targets in overweight and obese premenopausal women. Results are expected to contribute to a better understanding of those scientific fields of knowledge, providing a readily available technique to estimate thigh composition, along with the

identification of the associations between thigh components and maximal oxygen consumption, and PA, either in a cross-sectional as in a longitudinal approach, aiming the important context of obesity treatment.

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CHAPTER 2

General Methods

"Nothing has such power to broaden the mind as the ability to investigate systematically and truly all that comes under thy observation in life."

Marcus Aurelius

Introduction

A brief description of the methods used throughout the current Thesis is presented in this chapter. First, the sample will be described, and then the intervention design with the participating overweight and obese premenopausal women, followed by the body composition, maximal aerobic capacity, and PA assessment methods that were used. Finally, the statistical analysis employed will be described.

Sample

Participants were recruited from the community, in the greater Lisbon area, for a 2-year weight management program, through announcement flyers and poster information posted in health care facilities, a website, and email messages on listservs.

Participants Eligibility and Recruitment

To be eligible for study participation, women were required to be ≥25 years of age, BMI ≥25 kg/m^2 and ≤39.9 kg/m^2, pre-menopausal, without diagnosis of hypertension, dyslipidemia, diabetes, thyroide alterations, or other diseases, and medication that could influence body composition. Women could not be pregnant, not willing to become pregnant in the next 2 years, and had to be willing to be randomly assigned to either an intervention or comparison group. Exclusion criteria consisted of age younger than 25 years old, BMI lower than 25 kg/m^2 or higher than 39.9 kg/m^2, being post-menopausal, having any condition likely to increase risk from the intervention or to limit life span, presenting any condition that would likely affect the ability to conduct the intervention as designed, or taking any medication or had any medical condition likely to confound the assessment of body composition.

Eligibility Visits

Eligible women attended plenary sessions in which all the intervention procedures and study compliance were explained. If they were willing to participate, and agreed with the procedures, a demographic and clinical questionnaire was filled out, and afterwards were invited to attend four screening visits, during which eligibility was further assessed. Women participated with the sole incentive of benefiting from the intervention, without any financial compensation.

First baseline screening visit. An informed consent approved by the Ethical Committee of the Faculty of Human Movement, Technical University of Lisbon, was signed. Body composition was assessed after a 12-hour fast, with anthropometry, bioelectric impedance spectroscopy, air displacement plethysmography, and dual- energy X-ray absorptiometry. Questionnaires estimating habitual PA, psychosocial and quality of life parameters were administered during the first visit. A 4-day food record was assigned for return at the second visit.

Second baseline screening visit. Cardiorespiratory fitness was assessed, and the completion of the psychosocial questionnaires and of the 4-day food record was performed.

Third baseline screening visit. Biochemical assessments and body composition by CT were performed.

Final eligibility. For each woman, a clinical consultation with the intervention physician took place, in order to analyze the baseline results and decide if inclusion criteria were met, or if the subject should be excluded from the intervention.

Participants

Participants number varied in each study article because research requirements differed,

and only those women who completed all the assessments involved in each article were included in the analysis.

In **Study I (chapter 4)**, the sample comprised 110 pre-menopausal women (38.6±5.3 yr, 76.9±9.7 kg, 1.6±0.1 m, 30.0±3.2 kg/m^2). Participants who completed all initial measurements in **Study II in chapter 5** were 98 pre-menopausal women (38.2±5.5 y, 77.2±9.7 kg, 1.6±0.1 m, 30.1±3.0 kg/m^2). In **Study III (chapter 6)**, despite the fact that at sixteen months, after the intervention, 65 women were evaluated with CT, only 48 of them completed all the required assessments (39.2±5.7 y, 76.9±9.2 kg, 1.6±0.05 m, 30.0±3.0 kg/m^2).

Intervention Design

Studies I and II (chapters 4 and 5, respectively), were performed with baseline data, while **Study III in chapter 6** was carried out with data from the 16-month program. The intervention was empirically-based on the cognitive-behavioral approach to promote the adoption and maintenance of a healthy lifestyle, with a strong emphasis on PA and nutrition [1]. The lifestyle intervention goals were to: 1) achieve and maintain a weight reduction between 5%-10% of initial body weight through healthy eating and PA; 2) achieve and maintain a level of PA of at least 200-300 min/week (equivalent to ~1500-2000 Kcal/week), through moderate-intensity activity; 3) achieve and maintain a reduction in total energy intake (300-500 Kcal/day less), with a reduction on fat intake (<25% of total daily intake), an increase in complex CH (>45% of total CH daily intake), in fiber (>25 g/day), a daily protein intake of no more than 15% kcal/day, and the promotion of nutrient-dense, high-volume, low calorie foods such as fruits, vegetables and whole grains [1].

All the participants were submitted to a 16-week program, consisting of a 2-h weekly session, aimed at promoting lifestyle changes through education and behavioral strategies concerning nutrition, PA and lifestyle management, as described by others [2]. After this 16-week program, participants were randomized to a comparison group, monthly meetings group, and monthly meetings plus structured PA group. The design incorporates collection of outcome measures of all the participants at baseline, and at 16-week, and 16-month follow-ups.

Comparison Group

Each participant carried on with their life, away from the intervention team. Support from the intervention team could be asked by the women through telephone call or e-mail during the follow-up period.

Monthly Meetings Group

In this group participants benefited from a monthly meeting with the intervention staff, during which goal setting, self-monitoring, stimulus control, problem solving, relapse prevention, social support, and motivational techniques, concerning nutrition, PA and lifestyle management were addressed.

Monthly Meetings + Structured PA Group

Besides the monthly meetings, participants profited from 2 weekly sessions of structured exercise, performed between friday and sunday. The exercise program consisted of aerobic and strength training combined, to reach 300 kcal/session at the beginning of the program, progressing gradually to 400-500 kcal/session from 2-months on. Cardiovascular intensity was between 40%-50% HRR (heart rate reserve) when the intervention began, and progressed to 55%-70% HRR. The modified scale of perceived exertion [3] was used by the participants, aiming at a level between 4-8 of the scale. Strength training was incorporated, and the intensity was based on 1-RM assessment of

upper and lower-body. Large muscle groups were included, and different muscle groups were used in each session and between sessions.

Body Composition Assessment

Measurements of body composition using each technique were conducted according to standard procedures. Subjects came to the laboratory after a 12-hours fast, and 24-hours without exercise, alcohol or stimulant beverages. DXA and anthropometry measurements were carried out in the same morning, while CT was performed in a different day. In brief, the procedures were as follows.

Computed Tomography (CT)

To characterize thigh composition, regarding AT and SM tissue, within the thigh, an electron-beam cross-sectional helical CT imaging (Siemens, Somaton Plus) was performed, using the standard procedures as described by others [4, 5]. In **Studies I and II (chapters 4 and 5, respectively)**, contiguous 7-mm-thick cross-sectional images of both thighs were collected, between the inferior border of the ischial tuberosity and the superior border of the patella. The tissue volumes (cm^3) identified in each image were computed as the product of the tissue area (cm^2) by the image thickness (7 mm). Thigh SM volume (litters) was converted to mass units (kilograms) multiplying the volume by the constant density assumed for SM (1.04 kg/L) [6]. Thigh AT (litters) was converted to mass units (kilograms) multiplying the volume by the assumed constant density for FM (0.92 kg/L) [6]. An integrated three dimensional representation of thigh composition is showed in Figure 2.5. In **Study III (chapter 6)**, a cross sectional scan, at the medium distance between the inferior border of the ischial tuberosity and the

superior border of the patella, was selected to estimate the different compartments of thigh composition in each participant.

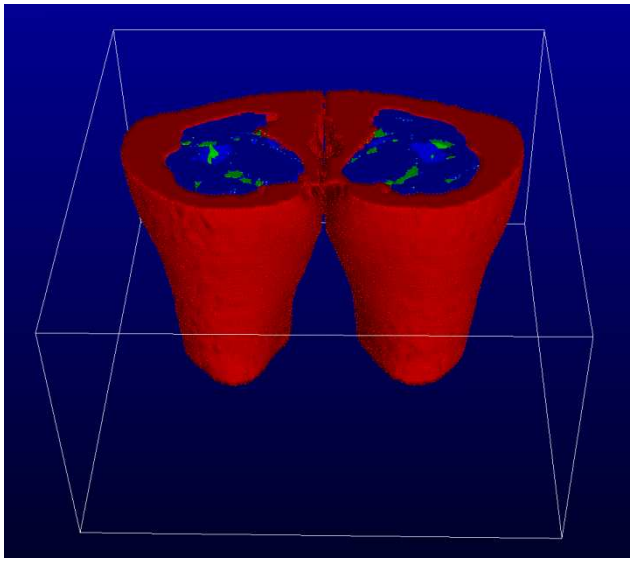

Figure 2.5. Integrated three dimensional representation of thigh composition showing SM tissue, TSAT and TIAT volumes.

Once accquired, the CT data were analyzed using commercially available software (Slice-O-Matic; Tomovision Inc., Montreal, Canada). A combination of edge detection filters and watershade techniques were used to distinguish different grey-level region on the slice images, corresponding to distinct tissues, which, after their identification, were tagged using different color codes [7]. The tissue segmentation was computed using standard HU ranges: -29 to +150 HU for SM, -190 to -30 HU for AT, and +152 to +1000 HU for bone tissue [7-9]. Normal density SM (+31 to +150 HU) and low density SM (-29 to +30 HU) were also measured [10]. Data correction was needed because the skin is hardly identifiable in CT images, and a thickness of 1mm was assumed [11].

All scans were acquired in one center under high levels of quality control by a well-trained examiner skilled in performing a protocol for body composition assessment. The reliability for thigh CT-measured SM and AT was calculated in 30 women. Only the intra-observer error was estimated due to the fact that the same technician made all segmentation measurements. The intra-observer analysis was performed on the same images separated by 3 months. The CV of test-retest reliability for mid-thigh SM tissue (cm^2) , total mid-thigh AT (cm^2), subcutaneous AT (cm^2), and intermuscular AT (cm^2) were 0.1%, 0.4%, 0.4% and 2.5%, respectively.

Dual-energy X-ray Absorptiometry (DXA)

FM, fat-free mass (FFM), and LST mass, in the whole body and in the lower limbs, were assessed by DXA (QDR-1500; Hologic, Waltham, MA, pencil beam mode, software version 7.2 enhanced whole body analysis). The lower limbs region was defined using the standard analysis protocol for the whole body scan, described by the manufacturer, and thigh regions of interest were identified, between the inferior border of the ischial tuberosity and the superior border of the patella. The same laboratory technician positioned the subjects, performed the scans, and executed the analysis according to the operator's manual. Based on ten subjects, the coefficients of variation in our laboratory for FM and FFM were 2.9% and 1.7%, respectively.

Anthropometry

In **Study I, Study II, and Study III (chapters 3, 4, and 5, respectively)**, body weight was measured with an electronic scale (BOD POD® Life Measurement Instruments, Concord, CA) to the nearest 0.1 kg, and height was obtained with the SECA scale stadiometer, measured to the nearest 0.5 cm. BMI calculated as kg/m^2, was defined as

the criterion to identify overweight and obese subjects in the sample, accordingly with the criterion of NIH and WHO [1, 12].

A trained researcher measured circumferences (waist, hip, proximal thigh, midthigh, and distal thigh) according to the procedures of others [1, 13]. Based on 10 repetitions, the technical error of measurement (TEM) and intraclass coefficient of correlation (ICC) were, respectively, 0.41 and 1.00 for waist circumference, 0.52 and 0.99 for hip circumference, 0.19 and 1.00 for proximal thigh circumference, 0.17 and 1.00 for midthigh circumference, and 0.37 and 0.97 for distal thigh circumference.

Maximal Aerobic Capacity

A breath-by-breath system was used to assess CRF in **Study II in chapter 4**, and in **Study III in chapter 5**. Airflow and volume were continuously measured and, simultaneously, instantaneous expired CO_2 and O_2 concentrations were also determined during exercise (MedGraphics® Corporation, utilizing BREEZEX Software). Maximal aerobic power (VO_2max), symptom-limited, was measured using an incremental protocol [14] on a motor-driven treadmill (Quinton Model 640 Treadmill Controller and Series 90TM Treadmills), with variable speed and grade, as described previously [15]. The criteria for VO_2max attainment included one of the following conditions: respiratory exchange ratio>1.1, plateau of VO_2 (change <100 mL/min in the last three 20-second intervals, or with an increased workload as evidenced by a difference in oxygen uptake of <2 mL/kg/min), a heart rate within 10 beats/min of age-predicted maximal heart rate, systolic blood pressure of 250 mm Hg or more, a decrease in diastolic blood pressure superior to 10 mm Hg, peripheral fatigue, or volitional exhaustion [16]. Reproducibility was not performed because this was a maximal test.

Physical Activity

Daily PA was objectively measured in **Study II (chapter 4)**, while in **Study III (chapter 5)** a self-reported questionnaire was used.

Objective Assessment of PA

At baseline, accelerometry method was used to objectively assess PA (CSA, model 7164, Shalimar, FL). This accelerometer was conceived to record uniaxial (vertical) acceleration of human movement, detected as a combined function of the frequency and intensity of the movement, with ranging magnitudes from 0.05 to 2 G. A 60-s epoch was defined for data storage, and in each count data PA intensity was recorded. Biological calibration of the CSA accelerometers took place before the assessment was performed. All the CSA accelerometers used in this study produced a response within the manufacturer's standards (± 5% of the reference value). The accelerometers that did not meet this criterion, or that revealed a broken beam or sensor unit, were excluded. Each CSA was placed on the right anterior axillary line at right iliac crest of the subject, secured tightly in nylon pouches, with velcro closures on an elastic waist belt, supplied by the manufacturer. The CSA activation was made according to standard specifications. Participants were instructed to use the CSA for the entire evaluation week [17], seven consecutive days, during the daytime, except while bathing and during aquatic activities. After that time period, data from the CSA was downloaded to a personal computer to be processed and cleaned, by using a macro written in Microsoft EXCEL (Microsoft Inc, Redmond, WA). Missing data, which were defined as sequences of ≥10 consecutive zero counts, were automatically deleted before analysis. The outcome variables were daily activity counts (counts/min/day), which is an indicator of the total volume of physical activity, and time (counts/min) spent at different physical activity-intensity categories: sedentary behavior (<100 counts/min),

light intensity activity (100 to 1952 counts/min), moderate intensity activity (1952 to 5724 counts/min), and vigorous intensity activity (>5724 counts/min). The cut-off points defined for each category were set accordingly to Freedson et al. scale [18].

PA Questionnaire

At baseline and 16-mnths, PA was assessed with the International Physical Activity Questionnaire [19], an instrument with a mean rho for criterion validity of about 0.30. The short last seven days self-administered format was used, considering its reasonable measurement properties for monitoring population levels of PA among 18- to 65-yr-old adults in diverse settings [19], and in special populations such as obese subjects [20]. Computation of the total score for the short form requires summation of the duration (in minutes) and frequency (days) of walking, moderate-intensity and vigorous-intensity activities [21]. The MET scores for each of the reported activities were derived using the Ainsworth et al. compendium [22], which classifies as moderate intensity activities those between 3 and 6 METs, and as vigorous intensity activities those greater than 6 METs. Reliability data were collected, and test-retest repeatability was performed within a two-week period.

Statistical Analysis

Measures of central tendency and distribution were examined in the three **Studies (chapters 3, 4 and 5)** for all outcome measurements, and are presented as mean±SD (standard deviation), alongside with normality tests for all variables, and for differences between baseline and 16-month assessments. In **Studies I, II and III**, multiple linear regression models were used: in **Study I (chapter 3)**, those statistical techniques were employed to develop SMHU, SM, TTAT, TSAT, and TIAT prediction equations, with total thigh CT as the dependent variables, and mid-thigh single slice SMHU, SM,

TTAT, TSAT, and TIAT, DXA and anthropometry estimates as the independent variables; in **Study II (chapter 4)**, the associations between CRF, PA, thigh SM mass, thigh SM attenuation characteristics, and thigh AT mass, were analyzed while controlling for age and BMI; in **Study III (chapter 5)**, multiple linear regression models were used to analyze the associations between changes in thigh composition variables, and alterations in other body composition variables, controlling for age and BMI.

The statistical *jackknife* procedure predicted residual sum of squares (PRESS) was used in **Study I (chapter 3)** to cross-validate the regression equation obtained in all the models [23]. The normality of the residuals and the correlation of the absolute residuals with the variables in the models were used to assess the adequacy of the final prediction models. Multicollinearity was evaluated by the analysis of the variance inflation factor for each independent variable [24]. Intercept and slope were tested. Hopkins [25] showed that the Bland-Altman method [26] produces an incorrectly systematic proportional bias in the relationship between two measures, when one has been calibrated against the other, even though no proportional bias exists. Accordingly to this author, only vertical error (error in predicting the criterion) is minimized around the regression slope, and positive proportional bias is due to the mathematical relation that occurs when a method is calibrated against a criterion using least-squares regression. Therefore, in **Study I (chapter 3)**, we used the Hopkins approach [25] which plots the residuals (prediction errors) against predicted scores, coming close to a standard Bland-Altman plot, except that predicted values are plotted on the x axis rather than the average of predicted and criterion scores.

In **Study II (chapter 4)**, a Z score for thigh composition was computed as a clustered risk factor resulting from the mean of the sum of the thigh SM mass (kg), total thigh SM

attenuation coefficients (HU), thigh subcutaneous and intermuscular AT mass (kg), individual Z scores. The Z score distribution was analyzed in tertiles of CRF, and in tertiles of daily PA, using one-way ANOVA with the post-hoc Bonferroni correction, to allow comparison of thigh composition risk profile among different CRF conditions and total daily PA levels.

In **Study III (chapter 5)**, changes from 0 to 16-months were analyzed with the paired samples *t*-test, and with the Wilcoxon signed ranks test when normality was not verified. Differences between intervention groups, at the beginning and at the end of the program, were tested using ANOVA (time x intervention group). At 16-months the analysis of covariance (ANCOVA) was completed with PA variables entered as covariates. Due to the number of subjects in the present sample (n=48), a bootstrap resampling analysis was performed to test for the specific indirect (or mediated) effects [27]. The mediation models used the intervention group as the independent variable, while changes in thigh composition variables (SM areas and quality, and AT areas) were tested as dependent variables. Age, BMI, and physical activity changes were tested as mediators. A resampling procedure (5000 bootstrap samples), via the bias corrected and accelerated (BCa) estimates and 95% confidence intervals to present the indirect effects significance were performed. If the BCa 95% interval confidence did not included zero, it can be concluded that there was a significant indirect effect (at alpha=0.05) [27].

The Statistical Package for Social Sciences (SPSS inc., 14.0 version, Chicago, IL, USA) was used to perform statistical analysis, and the MedCalc statistical software (MedCalc Software, Mariakerke, Belgium) was employed. Statistical significance was set as $P<0.05$.

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CHAPTER 3

Predictors of total thigh composition: DXA and anthropometry-based prediction models

"Learning is not attained by chance, it must be sought for with ardor and attended to with diligence."

Abigail Adams

Abstract

Objective: The aim of the present study was to develop and subsequently validate predictive equations for total thigh composition assessment, using the thigh composition estimations performed by total thigh CT scans as the reference, and with the developed equations based on DXA and anthropometry estimates as the main predictor variables, in overweight and obese women.

Methods: Subjects were 110 pre-menopausal women (38.6±5.3 yr, 76.9±9.7 kg, 1.6±0.1 m, 30.0±3.2 kg/m^2). Total thigh composition was assessed by CT and DXA was used to assess FM, FFM and LST. Anthropometric assessment was performed through the estimation of body circumferences at the hip, proximal thigh, and distal thigh. Multiple regression analysis was performed to develop prediction equations for SM mass, SM quality, TTAT, TSAT, and TIAT. PRESS statistic was used to test the validity of the developed models, and the Hopkins approach was used to test the agreement.

Results: SM and SMHU prediction models revealed no significant differences when compared with the reference method (mean difference=-0.01 kg and a mean difference=-0.03 HU, respectively, $P>0.05$ for both), while TSAT prediction equation produced an underestimation (mean difference=1.19 kg, $P<0.001$), and an overestimation was observed for TTAT (mean difference=-3.24 kg, $P<0.001$), and for TIAT (mean difference=-0.03 kg, $P<0.05$) when compared with the respective reference methods. In the agreement analysis for inter-individual differences, a range from -0.61 kg to 0.59 kg for SM, from -3.60 HU to 3.6 HU for SMHU, from -4.48 kg to -2.00 kg for TTAT, from -1.71 kg to 0.15 kg to TSAT, and from -0.31 kg to 0.24 kg for TIAT, were observed, with a confidence interval of 1.96.

Conclusions: New prediction models for the different compartments of total thigh composition were developed and internally validated in a sample of overweight and obese pre-menopausal women. Thigh composition assessment can be accomplished by using the developed models but caution should be taken when using and interpreting its results, namely in what concerns AT components.

Keywords: Body composition, thigh composition, computed tomography, prediction models, obesity.

Background

Estimation of different thigh components, namely SM, SM quality, TTAT, TSAT, and TIAT, is assuming increasing interest in recent years due to its diverse applications in physiology, nutrition, clinical medicine, and evaluation of treatments and interventions [1-4], reflecting multidisciplinary interests and the need for methods to estimate this body region composition. The most accurate *in vivo* methods of measuring total and regional body composition at the tissue-organ level are MRI and multi-scan CT [5, 6]. Although these two methods are used as reference standards, their application in clinical routine practice and body composition research is limited because of lack of access to instruments, analysis complexity, cost, and in the CT method radiation exposure. In order to go beyond these limitations several studies have used DXA as an alternative imaging approach for body composition assessment at the molecular level.

Four DXA models have been derived for estimating SM mass based on imaging methods, with two of them addressing total-body SM mass in healthy adults [7], and in children and adolescents [8], while the other two concentrate on lower limbs SM mass [9] and in regional SM mass assessment, including the thigh region [10]. Lee et al. [11] derived two anthropometric-based models for total-body SM mass, in independent samples of non-obese and obese subjects, using MRI as the reference method. However, none of these studies addressed specifically the population group of adult (25-49 yr) overweight and obese premenopausal women, with a BMI range between 25-39.9 kg/m^2, neither other body composition components beside SM were studied. The only study that developed total body SM prediction equations for obese subjects [11], validated those equations in a group of 80 obese subjects (39 men and 41 women), with a large SD for age, and obtained a high standard estimation error in both equations (2.9 kg and 3.0 kg). Additionally, only one of those studies [10] has used CT as the reference

method, and even in that case, image acquisition was performed with only four slices obtained in the thigh region, without any information about total thigh volume and composition. Along with SM, other thigh components are relevant to know in overweight and obese subjects. This population presents more low density SM tissue area than leaner persons. However, SM quality is poorer due to a greater lipid infiltration, which is more relevant in obese patients with T2DM [12, 13]. The lower SM attenuation coefficient is inversely and significantly correlated with insulin resistance after controlling for visceral adiposity and overall obesity [14], and is also associated with an increased risk of mobility loss in the elderly [3, 15].

Due to the inexistence of prediction equations for the different thigh components specifically addressed to overweight and obese women, to CT and MRI limitations regarding specially cost, radiation exposure and time, and the increasing availability of DXA instruments, the aim of the present study was twofold: to develop predictive equations for total thigh composition assessment (i.e., SM density, SM tissue mass, and AT mass); to validate the developed equations, using total thigh CT as the reference and DXA and anthropometry estimates as the main predictor variables, to be applied in overweight and obese women.

Experimental Methods

Participants

The sample comprised 110 pre-menopausal women (38.6±5.3 yr, 76.9±9.7 kg, 1.6±0.1 m, 30.0±3.2 kg/m^2). All subjects were informed about the research design and signed a consent form according to the regulations of the Ethical Committee of the Faculty of Human Movement, Technical University of Lisbon. All the subjects were screened for medical conditions that could affect body composition and those who did not met

screening criteria were excluded from the sample. A similar procedure was performed concerning PA habits using as criterion that participants were previously sedentary for at least 1 year, but in this case data was self-reported.

Body Composition Measurements

In agreement with the literature, CT was chosen as the criterion method [5, 6, 16, 17]. All subjects arrived for testing in the morning after a 12-hour fast. Additionally, participants were asked to refrain from exercise, alcohol and stimulant consumption 24 h prior to testing.

Anthropometry

Body weight was measured with an electronic scale (BOD POD® Life Measurement Instruments, Concord, CA) to the nearest 0.1 kg and height was obtained with the SECA scale stadiometer, measured to the nearest 0.5 cm. BMI calculated as kg/m^2, was defined as the criterion to identify overweight and obese subjects in the sample. This criterion was set accordingly with international organizations (NIH and WHO) guidelines for overweight and obesity, referred to as BMI over 24.9 kg/m^2 and 29.9 kg/m^2, respectively.

A trained researcher measured circumferences (hip, proximal thigh, and distal thigh) according to the procedures of Callaway [18]. Based on 10 repetitions, the technical error of measurement (TEM) and intraclass coefficient of correlation (ICC) were, respectively, 0.52 and 0.99 for hip circumference, 0.19 and 1.00 for proximal thigh circumference, and 0.37 and 0.97 for distal thigh circumference.

Computed Tomography

Helical CT imaging (Siemens, Somaton plus) was performed using standard procedures described elsewhere [19, 20]. Contiguous 7-mm-thick cross-sectional images of both thighs were collected between the inferior border of the ischial tuberosity and the

superior border of the patella. In each subject, a cross sectional scan at the medium distance between those two anatomical points was selected to measure the different compartments of thigh composition. The tissue volumes (cm^3) identified in each image were computed as the product of the tissue area (cm^2) by the image thickness (7 mm). Thigh SM volume (litters) was converted to mass units (kilograms) multiplying the volume by the constant density assumed for SM (1.04 kg/L) [21]. Thigh AT (litters) was converted to mass units (kilograms) multiplying the volume by the assumed constant density for FM (0.92 kg/L) [21].

Once accquired, the CT data were analyzed using commercially available software (Slice-O-Matic; Tomovision Inc., Montreal, Canada). Edge detection filters and watershade techniques were used to distinguish different grey-level region on the slice images, corresponding to distinct tissues, which, after their identification, were tagged using different color codes [5]. The tissue segmentation was computed using standard HU ranges: -29 to +150 HU for SM and -190 to -30 HU for AT [5, 22]. Normal density SM (+31 to +150 HU) and low density SM (-29 to +30 HU) were also measured [12]. Data correction was needed because the skin is hardly identifiable in CT images, and a thickness of 1 mm was assumed [23].

All scans were acquired in one center under high levels of quality control by a well-trained examiner skilled in performing a protocol for body composition assessment. The reliability for thigh CT-measured SM and AT was determined in 30 women. Only the intra-observer error was calculated due to the fact that the same technician made all segmentation measurements. The intra-observer analysis was performed on the same images separated by 3 months. The CV for mid-thigh SM tissue, and total mid-thigh AT were 0.1% and 0.4%, respectively.

Dual-energy X-ray Absorptiometry

DXA was used to assess FM and LST mass for both the whole body and specific regions (head, trunk, upper and lower limbs, and thighs). The equipment (QDR-1500; Hologic, Waltham, MA, pencil beam mode, software version 7.2 enhanced whole body analysis), measured the attenuation of X-rays pulsed between 70 and 140 kV synchronously with the line frequency for each pixel of the scanned image. Following the protocol for DXA described by the manufacturer, a step phantom with 3 fields of acrylic and 3 fields of aluminium of varying thickness and known absorptive properties was scanned alongside each subject to serve as an external standard for the analysis of different tissue composition. Based on ten subjects, the CV in our laboratory for FM and FFM were 2.9% and 1.7%, respectively. The same laboratory technician positioned the subjects, performed the scans and executed the analysis according to the operator's manual, using the standard analysis protocol for the whole body scan and a specific protocol to define thigh compartments. These regions of interest were identified between the inferior border of the ischial tuberosity and the superior border of the patella. All the subjects were analysed after a 12 hours fast.

Statistical Analysis

Multiple regression analysis was used to develop SMHU, SM, TTAT, TSAT, and TIAT prediction equations with total thigh CT as the dependent variable, and DXA and anthropometry estimates as the independent variables. Collinearity was tested in each prediction model.

The statistical jackknife procedure Predicted Residual Sum of Squares (PRESS) was used to cross-validate the regression equation obtained in all the models [24]. The PRESS statistic is an internal cross-validation procedure alternative to data splitting that

measures how well an equation performs when applied to independent samples. Validation using the PRESS procedure is similar to applying the equation to an independent sample because the PRESS residual is obtained for the observations that are not included in the data when the equation is derived [25]. This method is convenient when insufficient independent data are available, and has the advantage of providing a useful case diagnostic [24]. According to this procedure the following steps must be performed: 1. Fitting a regression equation with one observation left out of the model in turn; 2. Obtaining the predicted value of the excluded observation; 3. Calculating the residual for that predicted value (observed-predicted); 4. Repeating steps from 1 to 3 for all observations; 5. Obtaining the sum of squares (SS) of all residuals. The PRESS statistic replaces the ordinary residuals with PRESS residuals:

$$\text{PRESS} = \text{SS (PRESS residuals)}$$

This yields modified versions of R^2 and SEE:

$$R^2_{\text{PRESS}} = 1 - [\text{PRESS/SS (total)}]$$

$$\text{SEE}_{\text{PRESS}} = \sqrt{(PRESS / n)}$$

n – number of observations.

The normality of the residuals and the correlation of the absolute residuals with the variables in the models were used to assess the adequacy of the final prediction models. Multicollinearity was evaluated by the analysis of the variance inflation factor for each independent variable [26]. Intercept and slope were tested. Bland-Altman [27] recommended plot the error (Method 1-Method 2) against the average of the two methods to test for proportional bias. These authors demonstrated that this procedure is correct when considering the agreement of two existing methods, but Hopkins [28] showed that this method produces an incorrectly systematic proportional bias in the relationship between two measures, when one has been calibrated against the other,

even though no proportional bias exists. Accordingly to this author, only vertical error (error in predicting the criterion) is minimized around the regression slope, and positive proportional bias is due to the mathematical relation that occurs when a method is calibrated against a criterion using least-squares regression. Therefore, in the present study, we used plots of residuals (prediction errors) against predicted scores, which is similar to a standard Bland-Altman plot, except that predicted values are plotted on the x axis rather than the average of predicted and criterion scores. The Statistical Package for Social Sciences (SPSS inc., 14.0 version, Chicago, IL, USA) was used to perform statistical analysis and the MedCalc statistical software (MedCalc Software, Mariakerke, Belgium) was used to perform the Hopkins analysis, with statistical significance set as $P<0.05$.

Results

Participants Characteristics

One hundred and thirty five overweight and obese pre-menopausal women participated in this study, but only 110 completed the study protocol. Age was (mean±SD) 38.8±5.3 yr, with a range of 25-49 yr. The height of the subjects ranged from 1.5 m to 1.7 m, and was 1.6±0.1 m. Their weight was 76.9±9.7 kg, and was between 59.1 kg and 104.4 kg, while their BMI (30.0±3.2 kg/m^2) ranged from 25.1 kg/m^2 to 39.1 kg/m^2. Sample characteristics and measurements are described in Table 3.4.

Model Development

New models were developed for SMHU, SM, TTAT, TSAT, and TIAT, using the entire sample, as presented in Table 3.5.

Table 3.4. Subject characteristics of the whole group, with thigh composition results from CT and body composition estimated by DXA.

Measurement	Mean±SD	Range
N	110	--
Age (yr)	38.75±5.34	25-49
Weight (kg)	76.91±9.66	59.10-104.40
Height (m)	1.60±0.06	1.46-1.73
BMI (kg/m^2)	30.01±3.17	25.10-39.14
Hip_{Circ} (cm)	110.92±6.89	94.7-134.6
PrT_{Circ} (cm)	64.74±4.56	53.4-76.3
$DistalT_{Circ}$ (cm)	39.30±2.78	33.3-49.5
Total thigh CT		
SMHU (HU)	41.28±2.47	32.80-47.38
SM (kg)	5.99±0.78	4.43-8.21
TTAT (kg)	8.23±2.04	4.02-14.77
TSAT (kg)	7.71±1.97	3.75-13.93
TIAT (kg)	0.76±0.22	0.39-1.39
Body composition DXA		
FM (kg)	35.34±7.47	23.51-57.73
FM (%)	46.00±4.93	33.82-59.12
FM_{Ll} (kg)	12.85±3.08	5.74-22.28
LST (kg)	38.43±4.09	28.46-49.43
LST_{Ll} (kg)	12.29±1.63	8.76-16.96

BMI, body mass index; FM, total body fat mass estimated by DXA; FM_{Ll}, lower limbs fat mass; LST, total body lean soft tissue mass; LST_{Ll}, lean soft tissue of the lower limbs; SMHU, skeletal muscle Hounsfield units; SM, skeletal muscle mass; TIAT, thigh intermuscular adipose tissue mass; TSAT, thigh subcutaneous adipose tissue mass; TTAT, thigh total adipose tissue mass; %FM, relative fat mass.

The significant predictors of SMHU were age, total body FM, distal thigh circumference ($DistalT_{Circ}$), and the square of the product between hip circumference (Hip_{Circ}) and proximal thigh circumference (PrT_{Circ}). For the SM, the significant predictor variables were lower limbs LST (LST_{Ll}), and also the $DistalT_{Circ}$. TTAT was explained by the variables FM and lower limbs FM (FM_{Ll}), and the product between Hip_{Circ} and PrT_{Circ}. The prediction model of the TSAT included FM_{Ll}, and the square of the PrT_{Circ} (PrT_{Circ}^2). TIAT was explained by total body relative FM (%FM), Hip_{Circ}, and LST of the entire body (LST). The developed models were validated by the PRESS

statistic method. The accuracy of the TTAT and TSAT was excellent, and SM accuracy was good, as indicated by the high R^2, and lower SEE obtained from the PRESS statistic method, while TIAT and SMHU accuracy were considered fair (Table 3.5).

Table 3.5. Calibration models for SMHU, SM, TTAT, TSAT and TIAT.

Model	Predictor variables	β (95% CI)	AdjR^2	SEE	Cross-validation	
					R^2_{PRESS}	SEE_{PRESS}
SMHU (HU)			0.425	1.873	0.425	1.863
	Intercept	61.631[a] (54.567, 68.694)				
	Age	-0.102[b] (-0.172, -0.033)				
	FM	-0.203[a] (-0.279, -0.127)				
	$DistalT_{Circ}$	-0.318[b] (-0.519, -0.118)				
	$(Hip_{Circ}xPrT_{Circ})^2$	6.24E-008[b] (0.000, 0.000)				
SM (kg)			0.843	0.307	0.840	0.309
	Intercept	1.502[a] (0.674, 2.331)				
	LST_{Ll}	0.462[a] (0.420, 0.503)				
	$DistalT_{Circ}$	-0.030[c] (-0.054, -0.006)				
TTAT (kg)			0.945	0.479	0.941	0.493
	Intercept	-2.294[a] (-3.174, -1.413)				
	FM	0.027[c] (0.006, 0.048)				
	FM_{Ll}	0.436[a] (0.364, 0.509)				
	$Hip_{Circ}xPrT_{Circ}$	0.001[a] (0.000, 0.001)				
TSAT (kg)			0.944	0.465	0.943	0.468
	Intercept	-1.881[a] (-2.625 -1.138)				
	FM_{Ll}	0.479[a] (0.417, 0.540)				
	$(PrT_{Circ})^2$	0.001[a] (0.000, 0.001)				
TIAT (kg)			0.564	0.143	0.559	0.143
	Intercept	-1.388[a] (-1.840, -0.935)				
	%FM	0.039[a] (0.030, 0.047)				
	Hip_{Circ}	-0.009[b] (-0.016, -0.003)				
	LST	-0.036[a] (0.026, 0.046)				

Abbreviations: β, beta (regression coefficient); $DistalT_{Circ}$, distal thigh circumference; FM, total body fat mass estimated by DXA; $FM_{Appendicular}$, appendicular fat mass; FM_{Ll}, lower limbs fat mass; $Hip_{Circ}xPrT_{Circ}$, hip circumference multiplied by proximal thigh circumference; LST, total body lean soft tissue mass; $LST_{Appendicular}$, appendicular lean soft tissue mass LST_{Ll}, lean soft tissue mass of the lower limbs; PrT_{Circ}, proximal thigh circumference; R^2, adjusted coefficient of determination; SEE, standard error of measurement; R^2_{PRESS}, coefficient of determination using the PRESS method; SEE_{PRESS}, standard error of measurement using the PRESS method; %FM, relative fat mass.
[a]$p<0.001$; [b]$p<0.01$; [c]$p<0.05$.

Reference SM versus Predicted SM

SM assessment performed with the predicted model was not significantly different from reference SM (mean difference=-0.01 kg, $P>0.05$). Linear regression analysis shows that predicted SM explained 84% of the variance in the reference SM, with an estimated

error of 0.31 kg. The regression between predicted and reference SM differed significantly from the line of identity (P<0.001). On an individual basis, the differences between the reference and the predicted SM ranged from -0.61 kg to 0.59 kg, showing that error scores were homoscedastic. The negative and positive errors were evenly distributed around zero across the range of predicted SM scores (r=-0.003; P>0.05), indicating no proportional bias (Figure 3.6).

Reference SMHU versus Predicted SMHU

Predicted SMHU was not significantly different from reference SMHU (mean difference=-0.03 kg, P>0.05). Linear regression analysis shows that predicted SMHU explained 43% of the variance in SMHU, with an estimated error of 1.87 HU. The regression between predicted and reference SMHU differed from the line of identity (P<0.001). On an individual basis, the differences between the reference and the predicted SMHU ranged from -3.6 HU to 3.6 HU, revealing consistency in the spread of error scores across the range of predicted SMHU results. In addition, the lack of a slope (r=0.001; P>0.05) indicates no proportional bias in the predicted results (Figure 3.6).

Reference TTAT versus Predicted TTAT

The performance of predicted TTAT revealed an overestimation from the reference TTAT by 3.24 kg (P<0.001). Linear regression analysis shows that predicted TTAT explained 95% of the variance in reference TTAT, with an estimated error of 0.48 kg. The regression between predicted and reference TTAT differed significantly from the line of identity (P<0.001). In an individual basis, the differences between the reference and the predicted TTAT ranged from -4.48 kg to -2.00 kg, indicating the existence of inconsistency in the spread of the error scores across the range of predicted TTAT results. In addition, a negative slope was found between the difference of the methods

and the predicted results (ρ=-0.527; *P*<0.001), indicating that the developed equation produces error scores with a non-uniform distribution around zero, tending to a lower overestimation of TTAT in subjects with lower predicted TTAT, and to a greater overestimation in subjects with higher predicted TTAT (Figure 3.7).

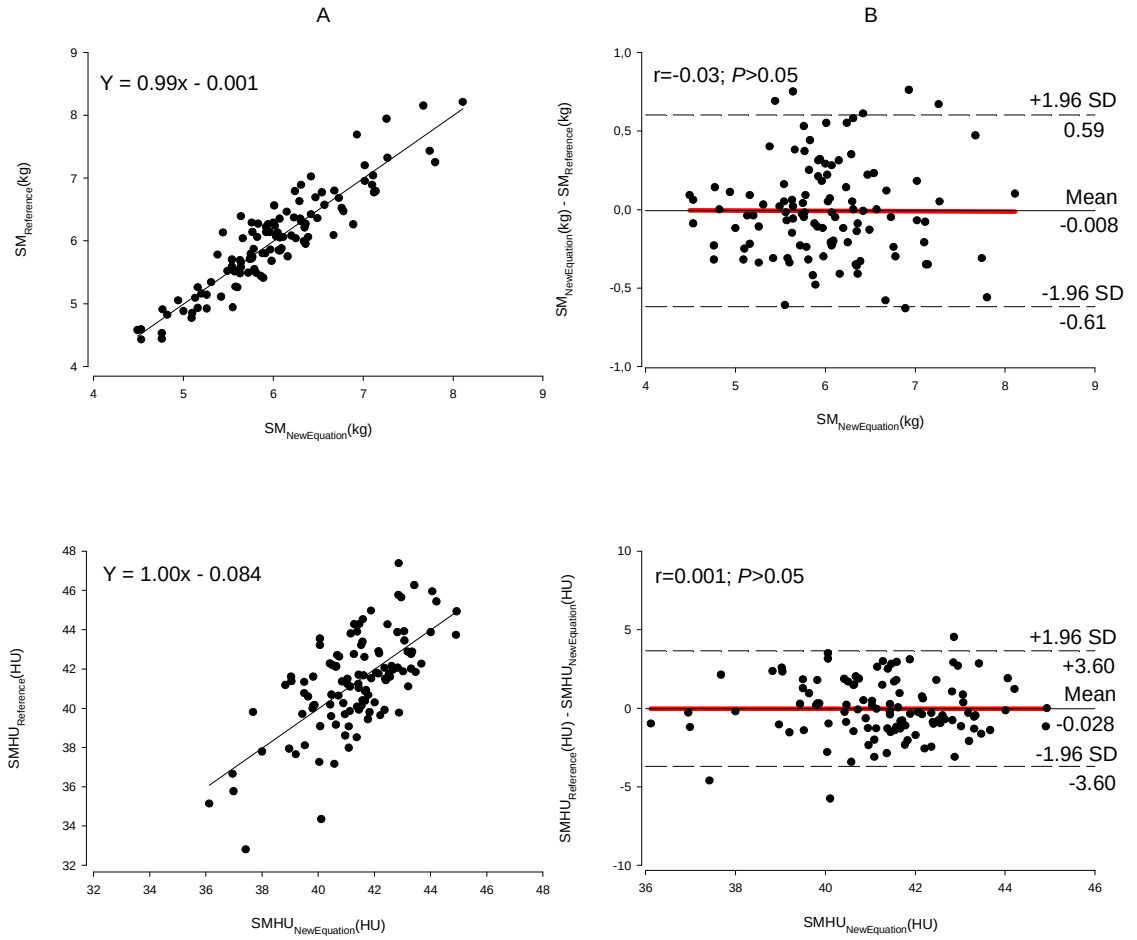

Figure 3.6. Linear regression for SM and SMHU estimation using the reference method (CT) and the respective calibration models with DXA and anthropometry variables (A) and regression between prediction errors against predicted scores (B). The solid lines represent the mean differences between the reference method and the developed equations, and the dashed lines represent (1.96 SD) confidence intervals (B).

Reference TSAT versus Predicted TSAT

Predicted TSAT was significantly different from reference TSAT ($P<0.001$), revealing an underestimation of 1.19 kg compared with the reference method. Linear regression analysis shows that predicted TSAT explained 94% of the variance in reference TSAT, with an estimated error of 0.46 kg. The regression between predicted and reference TSAT differed from the identity line ($P<0.001$). On an individual basis, the differences between the reference TSAT and the predicted TSAT ranged from -1.71 kg to 0.15 kg, indicating the existence of inconsistency in the spread of the error scores across the range of predicted TSAT results. A positive slope was found between the error scores and the predicted results ($r=0.355$; $P<0.001$), indicating that the new model produces error scores with a non-uniform distribution around zero, tending to a lower underestimation of TSAT in subjects with lower predicted TSAT, and to a greater underestimation in subjects with higher predicted TSAT (Figure 3.7).

Reference TIAT versus Predicted TIAT

The performance of predicted TIAT revealed an overestimation from the reference TIAT ($P<0.05$) by 0.03 kg. Linear regression analysis shows that predicted TIAT explained 56% of the variance in reference TIAT, with an estimated error of 0.14 kg. The regression between predicted and reference TIAT differed significantly from the line of identity ($P<0.001$). In an individual basis, the differences between the reference and the predicted TIAT ranged from -0.31 kg to 0.24 kg, showing that error scores were homoscedastic. The lack of a slope ($r=-0.013$; $P>0.05$) indicates no proportional bias in the predicted TIAT results (Figure 3.7).

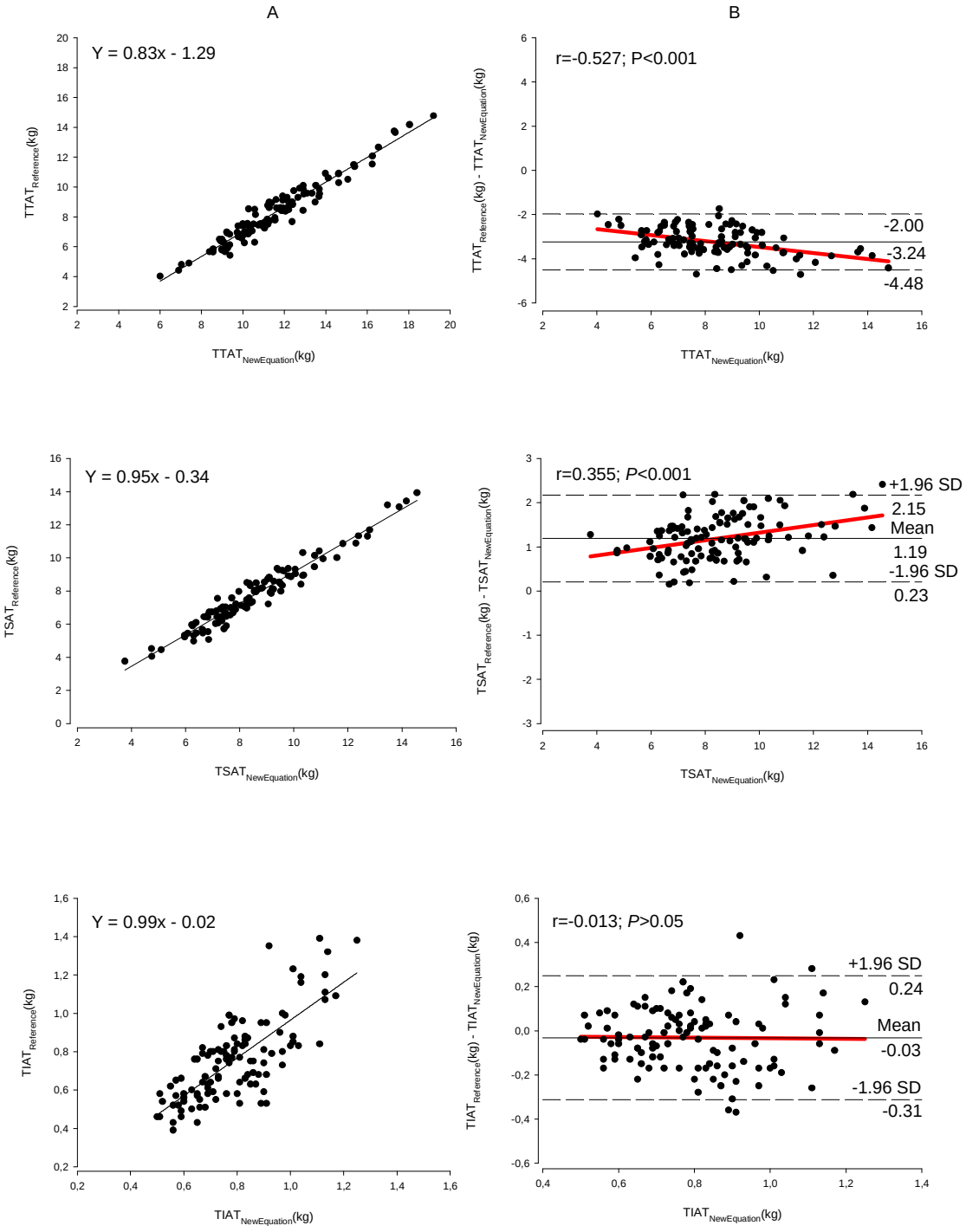

Figure 3.7. Linear regression for TTAT, TSAT and TIAT estimation using the reference method (CT) and the respective calibration models with DXA and anthropometry variables (A) and regression between prediction errors against predicted scores (B). The solid lines represent the mean differences between the reference method and the developed equations, and the dashed lines represent (1.96 SD) confidence intervals (B).

Discussion

To our knowledge, this is the first study that addresses the assessment of total thigh volume components by CT consecutive slices, through the development of models to apply when DXA and anthropometry are available, in a specific population group composed by overweight and obese premenopausal women. Due to DXA availability an its increasing use in body composition, the developed models obtained may be useful in different settings, such as exercise physiology, clinical medicine, nutrition, and during treatments and interventions. In the present study, SMHU and SM prediction models revealed no significant differences when compared with the reference method. The use of the developed equations produced an underestimation of ~15.4% for TSAT, while an overestimation of ~39.4% for TTAT, and ~3.9% for TIAT were observed. Better performance was found for SMHU and SM, specifically in an individual basis, showed by the unbiased agreement between the methods. Even so, an individual estimation error between ~(-8.7%) to ~(+8.7%) was found for SMHU, and between ~(-10.2%) to ~(+9.8%) for SM, when the prediction models were adopted in relation to the total mean value obtained with the reference method.

Model Development

New models were developed for SMHU, SM, TTAT, TSAT, and TIAT (Table 3.5). Age only explained part of the variance from the reference SMHU, which revealed a decrease with increasing age. SM quality of the total thigh was also negatively influenced by FM and by $DistalT_{Circ}$, indicating that the higher total body adiposity and the $DistalT_{Circ}$, the lower would be SM quality, in overweight and obese women. LST_{Ll} is the major contributor for SM. The negative association between SM and $DistalT_{Circ}$ indicates that women with more SM would present lower $DistalT_{Circ}$, reflecting less AT

accumulation in the thigh region. This last observation is in agreement with the positive association between TTAT and the result of the product between Hip_{Circ} and PrT_{Circ}, indicating that in overweight and obese women, AT distribution affected both hip and thigh circumferences. Direct associations were also found between TTAT with FM and FM_{Ll}, indicating that TTAT depot becomes more preponderant with increasing body fatness. The contributors to TSAT were FM_{Ll} and PrT_{Circ}^2, both with a positive influence. TIAT presented a direct association with %FM, and inverse associations with LST and Hip_{Circ}. These negative associations indicate that LST and Hip_{Circ} are lower in overweight and obese women with higher AT infiltration between muscle fibres.

CT and DXA Soft Tissue Validity and Accuracy

CT body composition methods were designed to quantify components at the tissue-organ level of body composition. In the limbs, the main components are AT, SM, and bone. Previous studies established the validity and accuracy of area and volume measurements from CT by comparing CT measures in human cadavers with direct measures (e.g., dissection, planimetry). A good agreement was found between cross-sectional area measurements of SM determined from the proximal thigh in cadavers, to the corresponding CT measured cross-sectional areas, with the correlation coefficient between the two approaching the unity [29], and between CT and cadaver AT areas, with correlations ranging from 0.77 to 0.94 [17]. A strong correlation was also found by Mitsiopoulos et al. [5] between cadaver and CT area measures for lean SM and subcutaneous AT in the arms and legs. DXA ability to estimate soft tissue is influenced by the variability in soft tissue composition, which is caused by the difficulty to distinguish between bone and soft tissue compartments, and also by the effects of hydration and tissue thickness [30]. A considerable inter-individual and within-subject

variability can be found in the density of FM and FFM, particularly in the proportion of water and mineral in FFM, affecting the accuracy of DXA estimates [31, 32], and contributing to the absolute error of this method. Food intake have only small effects on DXA estimates of body composition, with little change in bone mineral content or FM [33]. The variation of ±5% in the hydration status biases DXA estimates of %FM only 1% to 2.5% [34], but the ingestion of fluid and the regional accumulation or depletion of water and salts are significantly monitored by DXA as estimates of LST [33]. In what concerns overweight and obese subjects, an enlarged FFM hydration is observed [35]. AT consists of ~14% of water and has an extracellular/intracellular ratio (ECW/ICW) of ~3.7, while FFM consists of ~72% of water with an ECW/ICW of ~0.82, meaning that the more AT, the larger the relative contribution of ECW, which may explain the difference between non-obese and obese subjects [35], and also influence DXA estimates of FFM and FM. In the thigh region, this explanation is reinforced by the findings of Goodpaster et al. [13] who found a greater lipid infiltration within SM contributing to a decreased FFM density. In the current findings, predicted SM mass and SMHU showed a better accuracy than the other predicted variables, indicating an unbiased agreement between methods, while the lowest accuracy was observed for predicted TTAT and TSAT. These results suggest that in overweight and obese premenopausal women, the enlarged FFM hydration and the larger relative contribution of ECW might have a greater influence in DXA FM based estimates than in DXA LST based estimates. Several authors [34, 36] observed that DXA tends to overestimate FM, and an average higher error in obese subjects was also suggested, indicating that DXA is less accurate in subjects with larger FM [37]. DXA overestimation of FM may have contributed to the high overestimation observed in the present study with the TTAT developed model (~39.4%) when compared with the

reference method, as well as for the elevated individual variation observed for this variable (-54.4% to -24.3%). Another contributor to these results may be the fact that in the DXA technique pixels are graded as mixtures of lean tissue and fat tissue, differing from CT in which pixels are allocated to AT or lean tissue, depending on level of Hounsfield units. By providing the proportion of fat and lean in each pixel, DXA estimates assess fat rather than AT, unlike CT [38]. One of the strengths of the current study concerns the CT data collection method performed, composed of consecutive slices of the entire thigh, contributing to a more accurate data acquisition.

Model Utility

We elected to test the validity of using prediction models based on DXA and anthropometric variables to perform estimations compared to the CT reference method. Therefore, using these calibration models, it is possible to correct data obtained with DXA and anthropometry, and surpass the limitations of cost, radiation exposure, and analysis complexity inherent to CT equipments use in body composition assessment.

The SMHU and the SM developed models revealed no significant differences when compared with the reference method, while an underestimation was observed for TSAT, and an overestimation was obtained for TTAT and TIAT prediction models. Due to these results, caution is needed when using the new models for the AT components. Also, the current findings are only applicable with similar samples and DXA instruments (Hologic QDR-1500, pencil-beam-mode).

These models may be useful to analyze the associations between thigh composition and both muscle strength and metabolic indicators, especially when body composition in the clinical practice and sports settings is critical and need to be followed longitudinally. That could be the case of, for example, the measurement of the nutritionally important

SM compartment *in vivo* in wasting illnesses, such as in HIV, assuming a critical importance to allow the follow-up of the disease evolution. As obesity is a growing problem in HIV-positive women receiving highly active antiretroviral therapy [39, 40], considering a 30 year-old woman, with 1.65 m, 75 kg, with 12.3 kg of lower limbs LST, and a DistalT_{Circ} of 39.0 cm, she would have ~6.01 kg of SM in both thighs using the calibration model. If the goal for this woman was to increase 0,5 kg of SM, this would lead to an error of ~0.3 kg (~5%) that would affect the correct interpretation of thigh composition results and thus the medical therapy, nutritional and PA prescriptions for the clinical follow-up.

Clinical medicine may also benefit from thigh components estimation, by analyzing its associations with several disease states, such as insulin resistance, metabolic syndrome and obesity [1, 2, 4, 13, 41], and by evaluating treatments and interventions, including weight control [42], and sarcopenia [43]. The associations between SM and SM lipid content with incident mobility limitations, and lower extremity performance in the elderly [3, 15, 41, 44, 45] may also be enriched through this kind of data.

Study Limitations

There are several limitations of the present study. Considering the specific DXA instrument used, this study is of practical interest to a laboratory with the same model of equipment, because each manufacturer uses different detection, calibration and analysis techniques in their body composition assessments [46]. Even for the same manufacturer, results may vary with the DXA instrument model, the mode of data collection (eg, pencil-beam *versus* fan-beam), and the software used to analyse the data [46].

In what concerns CT data, a potential limitation involves the assumed constant density for SM and AT (1.04 kg/L and 0.92 kg/L, respectively), used to convert SM and AT

volumes to mass [21]. Variability in actual SM density may introduce an error in the CT-estimated SM mass and quality.

The present empirical calibration models were developed in a cross-sectional analysis, and it would be useful to establish the validity of these models in longitudinally-monitored samples, as well as with a larger sample that may allow the external cross-validation procedure. In addition, our developed models are based on a sample of overweight and obese pre-menopausal women, and therefore may not be accurate when applied to subjects with different characteristics, such as healthy subjects and patients with other health-related disorders.

Conclusions

In summary, these new prediction models for the different compartments of total thigh composition were developed and internally validated in a sample of overweight and obese pre-menopausal women. Thigh composition assessment can be accomplished by using the developed models for SM quality, SM mass, TTAT, TSAT, and TIAT, but caution should be taken when using and interpreting its results, especially in what concerns AT components. These models can be useful in clinical studies for body composition assessment in obesity, and its application in other health conditions such as wasting illnesses need to be tested.

Acknowledgements

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CHAPTER 4

Cardiorespiratory fitness and physical activity are associated with thigh composition determined by computed tomography in overweight and obese premenopausal women

"What is a scientist after all? It is a curious man looking through a keyhole, the keyhole of nature, trying to know what's going on."

Jacques Yves Cousteau

Abstract

Objective: The main purpose of this study was to analyze the associations between objectively measured PA and CRF with total thigh SM and AT composition determined by CT, in overweight and obese premenopausal women.

Methods: Participants were 98 women (38.2±5.5 y, 77.2±9.7 kg, 1.6±0.1 m, 30.1±3.0 kg/m^2). Electron-beam cross-sectional helical CT imaging (Siemens, Somaton Plus) was performed to characterize the location and amount of AT, along with SM tissue mass and quality within the thigh. Daily PA was assessed by accelerometry (CSA, model 7164, Shalimar, FL), and CRF was estimated by respiratory gas exchange collected (MedGraphics® Corporation, utilizing BREEZEX Software) during a maximum incremental exercise test, accordingly with the modified Balke protocol [1]. Multiple linear regression models were used to analyze the associations between PA and CRF and thigh SM mass, thigh SM attenuation characteristics, and thigh AT mass. A Z score (continuous variable) for thigh composition was computed, and was analyzed in tertiles of CRF, using one-way ANOVA with the post hoc analysis of Bonferroni to investigate the differences between tertiles.

Results: In the several models for thigh SM mass, besides BMI, CRF, expressed in mL/kg/min, emerged as the only association with this thigh tissue (β=0.09; *P*=0.001). Daily time spent in low intensity PA presented a negative association with TSAT tissue mass (β=-0.03; *P*=0.046), even when CRF was included in the model (β=-0.03; *P*=0.041). TIAT was negatively influenced by low intensity PA (β=-0.003; *P*=0.040), but when CRF was included in the model this association became marginally non-significant (β=-0.003; *P*=0.061). Inversely, daily minutes in sedentary behavior were positively associated with TIAT (β=0.003; *P*=0.031), however this association also became marginally non-significant after controlling for CRF (β=0.003; *P*=0.052). The

mean Z score of thigh SM composition revealed a graded increase across tertiles of CRF, with significant differences obtained in the Z score between the first and the third tertiles (P=0.026).

Conclusions: Current results suggest that in overweight and obese premenopausal women, CRF is associated with thigh SM quantity, while low-intensity PA appears to negatively influence AT. On the contrary, sedentary behavior positively influence thigh AT. In these women, maximal oxygen consumption level is associated with different thigh composition phenotypes. Present findings should be considered when developing and implementing weight control interventions, in order to allow the improvement of SM morphology via increased PA and CRF, which overall are known to have a positive impact on health-related risk factors.

Keywords: Body composition, thigh composition, computed tomography, cardiorespiratory fitness, physical activity, obesity.

Background

In the thigh region, CT allows the identification of SM volume and quality, as well as AT compartments, namely TSAT and TIAT [2-4]. Several studies have shown associations between thigh components and a number of health conditions [5-12]. Overweight and obese subjects present more low density SM tissue area than leaner persons; however, SM quality is poorer due to a greater lipid infiltration, which is more relevant in obese patients with type 2 diabetes [5, 6]. Insulin-stimulated glucose disposal is positively correlated with SM attenuation, determined by CT, in caucasian men and women, ranging from lean to obese (BMI 19.6-41.0 kg/m^2) [7], and with insulin resistance in obese non-diabetic asian-pacific subjects [8]. Lipid infiltration of SM is also related with an increased risk of mobility loss in the elderly [13, 14]. TIAT appear to be positively associated with the metabolic syndrome [10], and with cardiovascular disease risk factors [11], while TSAT seems to be related to the same variables but in the opposite direction [10-12].

Growing evidence has pointed to a possible correlative and causative relationship between physical inactivity and an increase of thigh fatness, and also a lower SM quality in adults [15, 16]. In older healthy adults PA level, measured by accelerometry, was inversely associated with the percentage of non-contractile tissue (mainly fat) in the leg, determined by MRI [17]. In patients with peripheral arterial disease, higher levels of accelerometry assessed PA, were associated with greater calf SM area and SM density, estimated by CT [18]. Muscle atrophy of the lower leg, obtained by MRI, was found to be associated with poor physical performance, determined through gait speed, in dialysis subjects compared with healthy sedentary controls [19]. Nevertheless, none of these analyses was performed with overweight and obese premenopausal women. Other studies have addressed cross-sectional area and composition of the lower body

skeletal muscles, and PA, either by accelerometry [20] or by questionnaire [21], but no association was reported between these variables.

CRF has been found to be inversely associated with midthigh low-density lean tissue, in women with a wide range of total body fat (10-55%) [22], but in postmenopausal women this correlation was not verified [23]; however, these authors found that midthigh SM area was positively correlated with maximal oxygen consumption. An increase in CRF was observed simultaneously with the increment of high-density SM area (cm^2), and of mean SM attenuation (HU), in lean and obese men with and without T2DM [24]. This was similar to the findings of Ross et al. [25], concerning the increase in SM within the exercise without weight loss group, in abdominally obese premenopausal women, and to those by Dolan et al.[26], with HIV-infected women who performed exercise. Nevertheless, none of these authors reported associations between thigh components and CRF.

Although PA and maximal oxygen uptake are advocated as important contributors to health, including obesity prevention and treatment, and considering the important role of AT and SM involvement, the association of PA and CRF with thigh composition is still unknown in obese women. Furthermore, only a few studies used the direct measurement of PA with accelerometry. Therefore, the main purpose of this study was to analyze the associations of objectively measured PA, and CRF with total thigh composition determined by CT, in overweight and obese premenopausal women.

Experimental Methods

Participants

Participants were recruited from the community for a 2-year weight management program through newspaper ads, poster information posted in health care facilities, a

website, email messages on listservs, and announcement flyers. To be eligible for the study, subjects were required to be older than 24 years, be premenopausal and not currently pregnant, be previously sedentary, have a BMI higher than 24.9 kg/m^2 and lower than 40.0 kg/m^2, as well as be free from major disease. An initial visit with the study physician, along with clinical testing, ensured that participants met all medical inclusion criteria. Participants who completed all initial measurements for the current study were 98 pre-menopausal women (38.2±5.5 y, 77.2±9.7 kg, 1.6±0.1 m, 30.1±3.0 kg/m^2). All participants were informed about the research design and signed a consent form approved by the Ethical Committee of the Faculty of Human Movement, Technical University of Lisbon.

Body Composition Measurements

Measurements were conducted according to standardized procedures. Participants came to the laboratory, after a 12-hour fast, and 24-hours without exercise, alcohol or stimulant beverages. All measurements were carried out in the same morning. In brief, the procedures were as follows.

Anthropometry

Body weight was measured with an electronic scale (BOD POD® Life Measurement Instruments, Concord, CA) to the nearest 0.1 kg, and height was obtained with the SECA scale stadiometer, measured twice to the nearest 0.5 cm (average was used). BMI calculated as kg/m^2, was defined as the criterion to identify overweight and obese participants in the sample. This criterion was set accordingly with international organizations (NIH and WHO) guidelines for overweight and obesity, referred to as BMI over 24.9 kg/m^2 and 29.9 kg/m^2, respectively.

*Computed Tomography*To characterize the location and amount of AT and SM tissue within the thigh, electron-beam cross-sectional helical CT imaging (Siemens, Somaton Plus) was performed, using the standard procedures described by others [27]. Scan parameters were set at 120kVp for 1 s, 360 mA, 512 X 512 matrix, with a 48 cm field of view. With the subject supine, 7-mm-thick cross sectional consecutive scans of both thighs were obtained in each subject, between the inferior ischial tuberosity and the superior border of the patella. The tissue volumes (cm^3) identified in each image were computed as the product of the tissue area (cm^2) by the image thickness (7 mm). Thigh SM volume (litters) was converted to mass units (kilograms) multiplying the volume by the constant density assumed for SM (1.04 kg/L) [28], and a similar procedure was used to convert thigh AT (litters) to mass units (kilograms), multiplying the volume by the assumed constant density for FM (0.92 kg/L) [28].

Once accquired, the CT data were analyzed using commercially available software (Slice-O-Matic; Tomovision Inc., Montreal, Canada) based on image morphology. A combination of edge detection filters and Watershed techniques was employed. Filters were used to distinguish different gray-level regions on the slice images, corresponding to diverse tissues. Once the tissue regions were identified, the observer could use the software to tag them by using colour codes. Tissue volume (cm^3) for each slice was calculated by multiplying the slice thickness by tissue area (cm^2). Tissue segmentation was made using standard HU ranges: AT (-190 to –30 HU), SM (-29 to +150 HU), and bone (+152 to +1000 HU) [29]. Because the skin is hardly identifiable in CT images, it was necessary to correct data, assuming that the skin had a thickness of 1 mm [28]. Thigh AT was further distinguished by manual tracing as: TTAT, TSAT and TIAT [30], of both the right and left thighs. Bone marrow AT was excluded from this analysis. All image analysis procedures were performed by the same technician. The CV of test-

retest reliability for mid-thigh SM tissue (cm^2), total midthigh AT (cm^2), subcutaneous AT (cm^2), and intermuscular AT (cm^2) were 0.1%, 0.4%, 0.4% and 2.5%, respectively.

Measurement of Cardiorespiratory Fitness

Respired gas volume, flow rates and respiratory gas exchange were collected during exercise (MedGraphics® Corporation, utilizing BREEZEX Software). A breath-by-breath system measured airflow and volume continuously and, simultaneously, instantaneous expired CO_2 and O_2 concentrations were also determined. The flow and volume were measured by a pneumotachograph calibrated with a serynge of 3 L (Hans Rudolph, inc.TM); for gas exchange measurements, the concentration of CO_2 and O_2 in the expired gas must be determined. The concentration of carbon dioxide was measured with an analyzer of infrared light (Type: NIDR, range 0-10%, response (0-90%): <100msec at 100cc/min, accuracy: ±0.1%); the concentration of oxygen was measured by electrochemical O_2 analyzer of Zirconia (range 0.01 to 99.99%, response (0-90%): <100msec at 100cc/min, accuracy: ±0.1%). An electrocardiograph of 12-leads was used (Quinton Q710, version 1.00) to produce an electrocardiogram reported continuously. These data allowed the quantification of the subject's cardiac limitation to exercise, and also heart rate stages and maximal heart rate. A maximum (symptom-limited) incremental exercise test was performed on a motor-driven treadmill (Quinton Model 640 Treadmill Controller and Series 90TM Treadmills) with variable speed and grade. The modified Balke protocol [1] was used to determine work capacity and maximal power in concretion work situation. After a warm up of 2 minutes at 1.2 mph, the treadmill was set at a speed of 3.4 mph and an initial grade of 0º during the first minute of exercise. Then, the treadmill speed was maintained constant throughout the entire exercise test. At the start of the second minute the grade was increased to 1º, and from

that on, an increase of 1º was performed at the beginning of every additional minute of exercise, until the participants were exhausted. The criteria for VO_2max attainment included 1 of the following conditions: respiratory exchange ratio>1.1, plateau of VO_2 (change <100 mL/min in the last three 20-second intervals or with an increased workload as evidenced by a difference in oxygen uptake of <2 mL/kg/min), a heart rate within 10 beats/min of age-predicted maximal heart rate, systolic blood pressure of 250 mmHg or more, a decrease in diastolic blood pressure superior to 10 mmHg, peripheral fatigue, volitional exhaustion [31]. In the present study, CRF was quantified as estimated VO_2max and expressed as liters per minute and as milliliters per kilogram of body weight. Participants were wearing sports clothes and shoes. Due to the fact that this was a maximal test, reproducibility was not performed.

Objective Assessment of Physical Activity

Accelerometry Method

The Computer Science and Applications, Inc. accelerometer (CSA, model 7164, Shalimar, FL), is a very small and lightweight lithium battery-powered accelerometer conceived to record uniaxial (vertical) acceleration of human movement, detected as a combined function of the frequency and intensity of the movement, with ranging magnitudes from 0.05 to 2 G. It stores data from frequencies between 0.25 to 2.5 Hz, allowing to filtering out movements outside the range of normal human movement, such as car vibrations. Data was stored in memory according to the defined 60-s epoch and in each count data was recorded the PA intensity. Biological calibration of the CSA accelerometers took place at the beginning and the end of the study. All the CSA accelerometers used in this study produced a response within the manufacturer's standards (± 5% of the reference value) (Computer Science and Applications). The CSA that did not met this criterion, revealed a broken beam or sensor unit, were excluded.

Each subject evaluated wore a CSA placed on the right anterior axillary line at right iliac crest, secured tightly in nylon pouches with velcro closures on an elastic waist belt, supplied by the manufacturer. The CSA activation was made according to standard specifications. Participants were instructed to use the CSA for the entire evaluation week [32], seven consecutive days, during the daytime except while bathing and during aquatic activities. After that time period, data from the CSA was downloaded to a personal computer to be processed and cleaned by using a macro written in Microsoft EXCEL (Microsoft Inc, Redmond, WA). Missing data, which were defined as sequences of ≥10 consecutive zero counts, were automatically deleted before analysis. The outcome variables were daily activity counts (counts/min/day), which is an indicator of the total volume of PA, and time (counts/min) spent at different PA-intensity categories. Time spent in sedentary behavior (<100 counts/min), in light activity (100 to 1952 counts/min), in moderate activity (1952 to 5724 counts/min), and vigorous activity (>5724 counts/min) was calculated. The cut-off points defined for each category were set accordingly to Freedson et al. (1998) [33] scale, and expressed in percentual values. It was also possible to determine the time that the monitor was worn, the number of minutes spent continuously in moderate activity (>1952 counts), and the number of 10 minutes periods spent at an intensity level superior to 1952 counts.

Statistical Analysis

Measures of central tendency, distribution, and normality were examined for all variables. Multiple linear regression models were used to analyze the associations between CRF, PA, thigh SM mass, thigh SM attenuation characteristics, and thigh AT mass. A Z score for thigh composition was computed as a clustered risk factor resulting from the mean of the sum of the thigh SM mass (kg), total thigh SM attenuation

coefficients (HU), TSAT and TIAT mass (kg), individual Z scores. Due to the fact that TIAT represent an unhealthier phenotype [5], its inverse value (1/x) was computed in order to be included in the Z score calculation. The Z score distribution was analyzed in tertiles of CRF, and in tertiles of daily PA, to allow the comparison of thigh composition risk profile among different CRF conditions and diverse PA levels. One-way ANOVA with the post-hoc Bonferroni correction was used to analyze the differences between tertiles. Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS inc., 15.0 version, Chicago, IL) and MedCalc Statistical Software (MedCalc Software, Mariakerke, Belgium). Statistical significance was set as $P<0.05$.

Results

Subject characteristics for the sample, including mean, SD, minimum, and maximum values of the independent and dependent variables, are presented in Table 4.6.

The women were obese (mean±SD, 30.1±3.0 kg/m^2), and abdominally obese (91.0±7.7 cm), as shown in Table 1. The participants were characterized by a wide variation in thigh SM mass, ranging from 4.4 to 10.3 kg, with the low values of thigh SM attenuation coefficients indicating the lipid infiltration of SM mass (41.0±2.7 HU). High amounts of TTAT (8.3±1.9 kg), and TSAT (7.9±2.0 kg) were observed, revealing greater mean values than those presented by SM tissue (6.1±1.0 kg). TIAT mass was the smallest AT compartment in this body region (0.8±0.2 kg). Total daily PA measurement results revealed that subjects spent the majority of time in sedentary behavior (144.8±12.9 min/d), while low intensity activities occupied between 29.2 to 86.8 min/d of their time. Moderate and vigorous intensity activities only contributed with a small amount to total daily PA (5.0±2.8 min/d and 0.03±0.05 min/d, respectively). These

results are in agreement with participant's very low CRF level (24.7±4.0 mL/kg/min), as presented in Table 4.6.

Table 4.6. Subject characteristics.

	Total Sample	
	X±SD	Range
Anthropometric measurements		
Age (years)	38.2±5.5	25-49
Weight (kg)	77.2±9.7	59.1-103.3
Stature (m)	1.6±0.1	1.5-1.8
BMI (kg/m^2)	30.1±3.0	25.1-37.1
Waist circumference (cm)	91.0±7.7	82.5-127.3
Cardiorespiratory fitness		
VO_2max (mL/kg/min)	24.7±4.0	9.5-36.6
Objectively daily physical activity		
Daily activity (counts/min/day)	393.3±123.9	98.7-838.1
Sedentary behavior (min/d)	144.8±12.9	112.5-175.3
Low intensity PA (min/d)	54.6±12.2	29.2-86.8
Moderate intensity PA (min/d)	5.0±2.8	0.01-13.9
Vigorous intensity PA (min/d)	0.03±0.05	0.00-0.30
CT thigh composition measurements		
Thigh SM tissue mass (kg)	6.1±1.0	4.4-10.3
Thigh SM tissue density (HU)	41.0±2.7	32.7-46.3
TTAT mass (kg)	8.3±1.9	4.4-14.2
TSAT mass (kg)	7.9±2.0	4.0-13.4
TIAT mass (kg)	0.8±0.2	0.4-1.5
Z score thigh composition	-0.0003±0.6	-1.4-1.4

AT, adipose tissue; BMI, body mass index; CT, computed tomography; HU, Hounsfield Units; PA, physical activity; SD, standard deviation; SM, skeletal muscle; TIAT, thigh intermuscular adipose tissue; TSAT, thigh subcutaneous adipose tissue; TTAT, total thigh adipose tissue; VO_2max, maximal oxygen consumption; X, mean.

Results for the regression models that used thigh composition compartments as the outcome variable are shown in Table 4.7. Each model included age (data not shown) and BMI as covariates. In the several models for thigh SM mass, besides BMI, CRF, expressed in mL/kg/min, emerged as the only predictor of this thigh tissue, even after controlling for PA variables (low intensity PA –Model 5, and sedentary behavior – Model 6). No associations were found between PA variables and SM tissue (P>0.05), as shown in Models 3 and 4. Thigh SM quality and TTAT mass did not show any association with CRF and PA variables (P>0.05).

Table 4.7. Multiple regression models examining the associations between thigh composition and CRF, and objectively measured daily PA, controlling for age and BMI, in pre-menopausal, overweight and obese women.

Variables	Model 1	Model 2	Model 3	Model 4	Model 5	Model 6
Thigh SM (kg)						
BMI (kg/m^2)	0.092**	0.140***	0.091**	0.090**	0.142***	0.141***
CRF (mL/kg/min)		0.090**			0.091**	0.091**
Low Intensity PA (min/d)			0.003		-0.002	
Sedentary Behavior (min/d)				-0.005		0.001
Thigh SM Quality (HU)						
BMI (kg/m^2)	-0.435***	-0.407***	-0.446***	-0.446***	-0.424***	-0.427***
CRF (mL/kg/min)		0.053			0.040	0.033
Low Intensity PA (min/d)			-0.022		0.020	
Sedentary Behavior (min/d)				-0.025		-0.023
TTAT (kg)						
BMI (kg/m^2)	0.432***	0.430***	0.442***	0.438***	0.447***	0.444***
CRF (mL/kg/min)		-0.004			0.009	0.010
Low Intensity PA (min/d)			-0.019		-0.019	
Sedentary Behavior (min/d)				0.015		0.015
TSAT (kg)						
BMI (kg/m^2)	0.423***	0.426***	0.436***	0.431***	0.450***	0.445***
CRF (mL/kg/min)		0.006			0.024	0.024
Low Intensity PA (min/d)			-0.026*		-0.027*	
Sedentary Behavior (min/d)				0.019		0.021
TIAT (kg)						
BMI (kg/m^2)	0.043***	0.040***	0.045***	0.045***	0.043***	0.043***
CRF (mL/kg/min)		-0.006			-0.004	-0.004
Low Intensity PA (min/d)			-0.003*		-0.003	
Sedentary Behavior (min/d)				0.003*		0.003

AT, adipose tissue; BMI, body mass index; CRF, cardiorespiratory fitness; PA, physical activity; SM, skeletal muscle; TIAT, thigh intermuscular adipose tissue; TSAT, thigh subcutaneous adipose tissue; TTAT, total thigh adipose tissue;
*** P<0.001; ** P<0.01; * P<0.05.

A negative association was found between daily time spent in low intensity PA and TSAT mass (Model 3), and this association prevailed when CRF was included in the model (Model 5). Minutes per day spent in sedentary behavior were marginally associated with TSAT (β=0.02; P=0.098), when controlling for CRF (Model 6). Low intensity PA was inversely associated with TIAT (Model 3), but when CRF was included in the model this association became marginally significant (β=-0.003;

P=0.061), as can be observed in Model 5. Although daily minutes in sedentary behavior were positively associated with TIAT mass (Model 4), this association became marginally significant when controlling for CRF (β=0.003; *P*=0.052), as presented in Model 6.

Figure 4.8 shows the mean Z score (continuous variable) of thigh SM and AT composition for tertiles of CRF, with the lowest Z score results indicating a poor thigh composition.

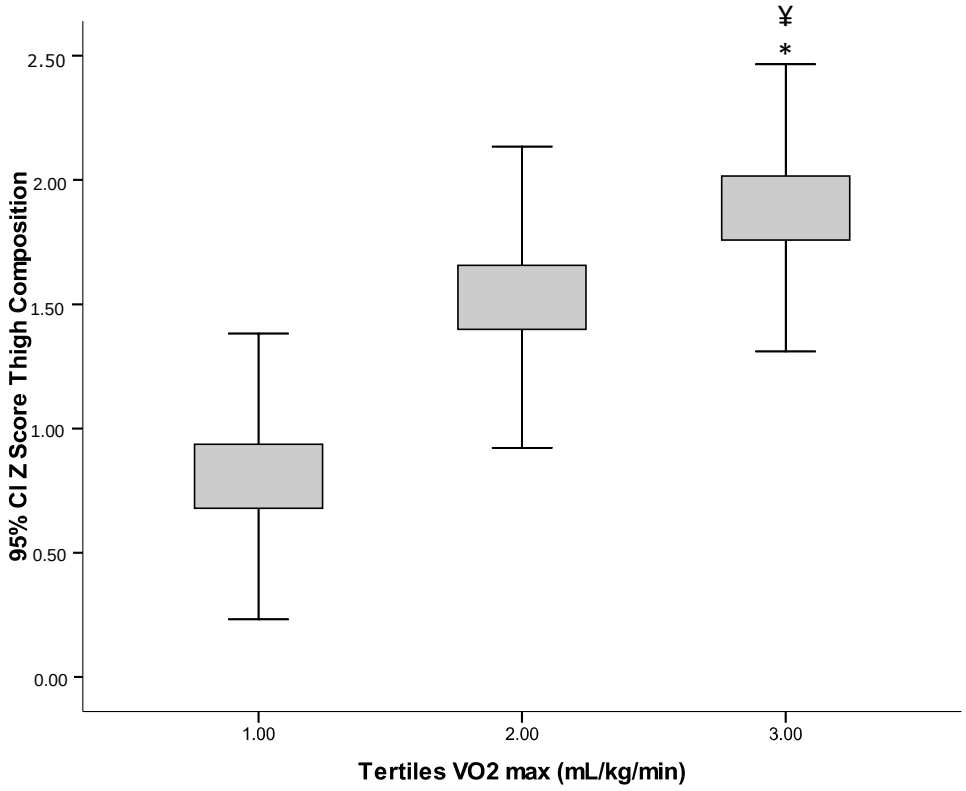

Figure 4.8. Mean Z score (continuous variable) of thigh composition for tertiles of maximal oxygen consumption (mL/kg/min). One-way ANOVA with the post-hoc analysis of Bonferroni were used for the comparison of means between tertiles (1^{st} *vs* 3^{rd}, * *P*<0.05; 2^{nd} *vs* 3^{rd}, ¥ *P*=0.061).

A graded increase was noted across tertiles of CRF, expressed in ml/kg/min, with significant differences obtained between the first and the third tertiles (*P*=0.026), and a marginally significant difference between the second and the third tertiles (*P*=0.061).

A similar increasing trend was verified for thigh composition across tertiles of total daily PA, but with no differences between tertiles (P>0.05), as shown in Figure 4.9.

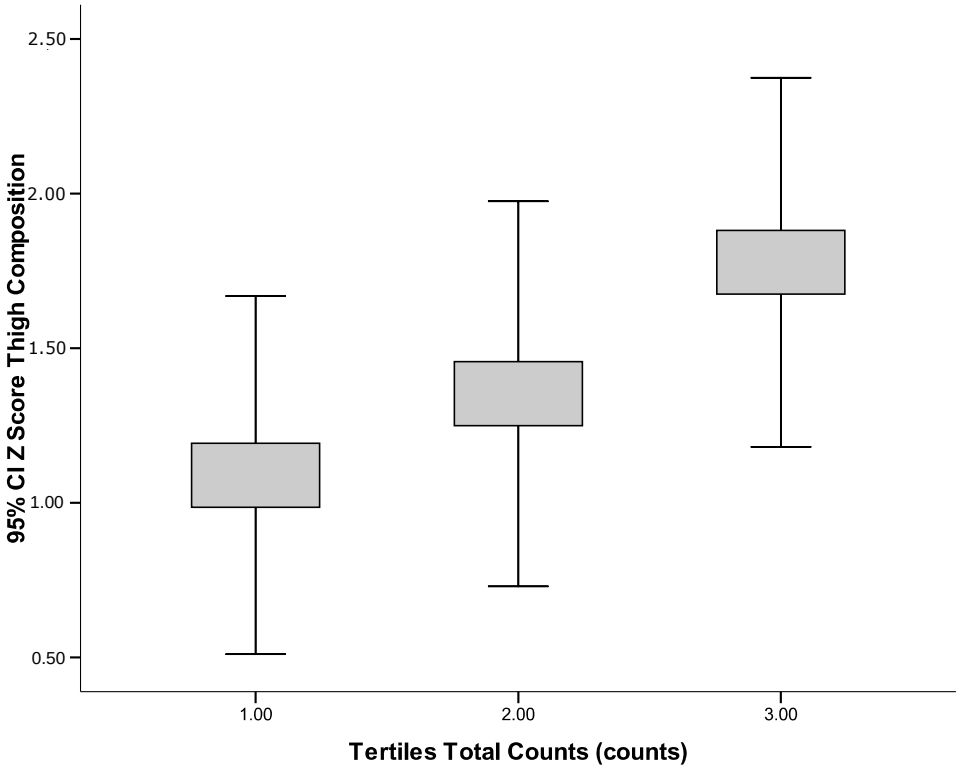

Figure 4.9. Mean Z score (continuous variable) of thigh composition for tertiles of total daily PA (total counts). One-way ANOVA with the post-hoc analysis of Bonferroni revealed no differences between tertiles (P>0.05).

Discussion

This study relied on thigh composition assessment through CT, PA objectively measured with accelerometers, and CRF estimation by standard procedures to investigate the associations between dependent (thigh composition) and independent variables (PA and CRF), in overweight and obese premenopausal women.

For daily PA, and considering the Freedson et al. (1998) [33] cut-off points to distinguish different PA-intensity categories, the analysis of total daily PA revealed that the sample spent the greatest amount of time in sedentary behavior, while low intensity activities occupied 26.6% of their time. Moderate and vigorous intensity activities only

had a very small contribution to total daily PA. As women in the present study were previously sedentary, their moderate and vigorous-intensity activities were well below the target of at least 200 min/week, recommended for weight loss [34], or 60-90 min of moderate-intensity activities per day for weight maintenance after weight-loss [35], or even at least 30 min of moderate-intensity PA, no less than 5 d/week, in order to prevent against chronic diseases [36]. These activity levels are not surprising since several studies have shown that BMI is inversely associated with PA of at least moderate-intensity in overweight and obese individuals, on the basis of recorded accelerometry weekly counts [37-39].

The higher percentage of time spent in sedentary behavior revealed by women in the present study may be partially a function of the Freedson et al. (1998) [33] cut points used. Ainsworth et al. (2000) [40] observed that the moderate-intensity cut point of Freedson et al. [33] (1952 counts/min) was higher than those of other authors [41, 42], and that it may be set too high to capture a broad range of moderate activity. Therefore, it is possible that Freedson et al's (1998) cut points may lead to a different classification for some moderate activities [40], precisely in the range of interest for public health recommendations concerning PA. Therefore, the results of the current study may underestimate moderate-intensity PA and overestimate time spent in low-intensity PA of these overweight and obese premenopausal women.

Activity levels for the participants are also in agreement with their CRF level, corresponding to a lower than average result [31], considering the age group of these women (25-49 years). The present results compare negatively with those obtained by Farrell et al. [43], which showed a maximal oxygen consumption in women associated with all-cause mortality in the Aerobics Center Longitudinal Study. Additionally, women with a moderate CRF for a given BMI or waist circumference have less total

FM and CT abdominal subcutaneous and visceral AT than individuals with low CRF, as shown by Janssen et al. [44]. These results may contribute to increase health risk factors in the present sample.

The present findings indicate that, in sedentary overweight and obese premenopausal women, although daily PA was not related with thigh SM quantity, a positive association may exist between CRF and SM mass, even after including PA as a covariate. Similar findings were observed in the Ryan et al. study [23], in which CRF was positively correlated with midthigh SM area (cm^2) in obese postmenopausal women, highlighting the importance of the association between maximal oxygen consumption and SM mass. Time spent in low intensity PA emerged as the main predictor (with BMI) of TSAT, with an inverse relation after controlling for CRF. Concerning TIAT, a negative association was also observed with minutes per day in low intensity PA, but this association became marginally significant after CRF was included as a covariate. Sedentary behavior was positively related with TIAT, but became also marginally significant after the inclusion of CRF. Current results suggest that in overweight and obese premenopausal women, time spent in sedentary behavior may induce a negative thigh composition profile, promoting the increase of intermuscular adiposity, while low intensity PA is associated with lower thigh AT deposition. No association was found between CRF, PA variables, and TTAT mass. Similarly, in a study with postmenopausal women, Ryan et al. [23] reported no associations between maximal oxygen consumption and midthigh AT. The absence of associations between CRF and daily PA variables with thigh SM quality may be due to the fact that only previously sedentary women were included in the sample. Due to the fact that the participants were previously sedentary, their daily PA and exercise may not represent an effective stimulus to induce SM tissue density modifications, which may

require more intense activities, and, consequently, fitter participants in order to be achieved. Other authors [23, 24, 26] demonstrated the positive effects of different exercise regimens on thigh SM quality of participants with diverse health conditions.

The low values obtained for thigh SM attenuation coefficients suggest lipid infiltration of SM mass, indicative of poor SM quality in this overweight and obese women. Other studies [21, 45], reported lower mean attenuation values, obtained with CT at midthigh, in older women when compared with the results of the present study, suggesting a decrease in SM quality with increasing age. Inversely, current results are somewhat lower than those observed by Lee et al. [24], who found a mean SM attenuation of 51.3±2.0 HU, in two continuous CT slices at the midthigh, in a sample of obese men, suggestive of a gender difference in thigh lipid infiltration. In middle-aged Korean women, Kim et al. [8] reported that the portion of the midthigh SM composed by low density SM area (+0 to +30 HU), seemed to be a determinant of insulin resistance, while Sakkas et al. [20] observed a positive association between T2DM and lipid deposition within SM of dialysis patients, estimated by MRI. This may suggest that overweight and obese women from the present study could be at a greater risk for the development of insulin resistance.

The analyses of clustered thigh composition-risk score for tertiles of maximal oxygen consumption revealed that women with the highest CRF presented a superior Z score, representing higher thigh SM mass and better SM quality, along with lower AT accumulation, than women with lowest CRF. This is important to note because this association is present despite the very low average CRF of study participants, indicating that, even in previously sedentary overweight and obese women, maximal oxygen consumption level is associated with different thigh composition phenotypes. The positive trend observed for the Z score across tertiles of total daily PA, suggests that

lower SM mass and density, and greater thigh adiposity may be associated with less time spent in PA in the daily routine.

The major limitation of the present study resides in its cross-sectional design, which does not allow establishing a cause-and-effect relationship between thigh composition and PA or CRF measures. Future research with follow-up approaches is needed in order to verify or infirm present findings. One of the strengths of this study was the assessment of PA with accelerometry, which allows to objectively quantify PA data in terms of time and intensity [38], information which can then be analyzed to examine patterns of activity over the course of several days or weeks [40]. These monitors provide the most objective and detailed record of PA for behavioral and epidemiologic research [46], and CSA monitors, in particular, appear to have good reliability for most research applications [47]. Another strength of the present study is the CT data collection method, which was composed of consecutive slices of the entire thigh, contributing to a more accurate data acquisition. Although multi-slice imaging is generally considered the reference for measuring total and regional AT and SM tissue [48, 49], most studies have used a single slice at the midthigh; this highlights the uniqueness of our study, but also difficulties in comparing the present results with previous findings. Nevertheless, caution is needed when comparisons between studies are made because different protocols might produce diverse results that should not be attributed to real discrepancies.

Conclusions

The results of the current study suggest that in overweight and obese premenopausal women, CRF is positively associated with thigh SM quantity, represented by SM mass

estimated from CT scans, while AT is negatively influenced by low-intensity PA. SM quality, represented by SM attenuation coefficients, possibly require more intense PA stimulus in order to reduce SM lipid infiltration. Nevertheless, it is important to note that, in these women, maximal oxygen consumption level is associated with different thigh composition phenotypes. The present findings should be considered when developing and implementing weight control interventions, leading to the improvement of SM morphology via increased PA and CRF, which overall are known to have a positive impact on health-related risk factors.

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CHAPTER 5

Impact of a weight-loss intervention on thigh composition, cardiorespiratory fitness, and daily physical activity: possible athlete's paradox effect?

> Although nature commences with reason and ends in experience it is necessary for us to do the opposite, that is to commence with experience and from this to proceed to investigate the reason.
>
> *Leonardo da Vinci*

Abstract

Objective: The current study analyzed the impact of a 16-month weight-loss intervention on thigh composition (SM area, SMHU, and AT area), CRF, and PA, in 48 overweight and obese premenopausal women, alongside with the investigation of the associations between changes in the studied variables.

Methods: Body composition was assessed (CT, DXA, and anthropometry). IPAQ was used, and CRF was tested. All measurements were performed at baseline and 16-months.

Results: Changes revealed increases in low density SM and thigh intermuscular AT (TIAT) areas, as well as for CRF and PA (P<0.001 for all). Decreases were observed for low density SM quality, TAT and thigh subcutaneous AT (TSAT) areas, anthropometric variables, and lower limbs FM (P<0.001 for all). No differences were observed between groups (P>0.05), even after adjustment for PA. The increase of low density SM area and the decrease of SMHU were inversely associated with the increment in CRF (P≤0.001 and P<0.05, respectively), and positively related with the reductions of weight (P≤0.001 and P<0.05, respectively), BMI (P≤0.001 and P<0.05, respectively), and others body composition indicators. TAT and TSAT decreases along with TIAT increment during the 16-months were positively associated with reductions in weight, BMI, anthropometric variables and lower limbs FM (P≤0.01 for all).

Conclusions: A 16-month weight-loss intervention positively influenced low density SM thigh area along with a decrease in low density SM quality and increases in CRF, suggesting the occurrence of the athlete's paradox in overweight and obese premenopausal women.

Keywords: Obesity, body composition, computed tomography, exercise.

Background

Obesity has rapidly become a global health problem [1, 2], and Portugal is no exception [3]. Moreover, obesity is a major cause of morbidity and mortality from coronary artery disease, cerebrovascular, hypertension, and T2DM complications [4-6]. Recent research is demonstrating associations between different body segments composition and those disease states [7-10]. These studies were made possible by technological advances in body composition methods, such as CT, and MRI. CT estimates of thigh composition are gaining more interest due to the associations found between AT and SM located in the thigh and several health conditions such as T2DM [11, 12], blood glucose and lipid levels [13], metabolic syndrome [8], prothrombotic and atherosclerotic abnormalities [14], and increased risk of mobility loss in the elderly [15].

Epidemiologic and experimental studies indicate that PA reduces risk factors for several health problems [16-21], with special emphasis for the prevention and treatment of obesity [22-25]. However, the role of PA in daily routine is still difficult to measure in free-living subjects [26, 27]. There are already some intervention studies with low density SM area and quality in the elderly [28], revealing the preservation of TIAT and of the SM attenuation value in the midthigh, in the PA group, while the controls showed an enlargement of both AT indicators. Few studies have addressed, simultaneously, PA estimation with questionnaires and lower limbs assessment by CT [29], and no studies were found addressing PA assessment with IPAQ and thigh composition with CT in overweight and obese premenopausal women.

CRF is an important health-related fitness indicator, which can be assessed through exercise testing, offering the unique opportunity to study simultaneously the cellular, cardiovascular, and ventilatory system responses under work conditions of precisely controlled metabolic stress. In different health conditions, including weight loss, and in

both genders, it was observed that subjects who performed exercise presented an increase in CRF along with a significant improvement in CT and MRI-measured SM quantity and quality (HU), at midthigh [30-34]. But, none of these studies was performed with overweight and obese premenopausal women, and none of these authors reported associations between thigh SM and thigh AT with CRF.

Despite CT availability, is still not known the effects of a long weight-loss intervention on the different thigh composition compartments, in obese premenopausal women. In addition, associations between thigh composition changes and modifications of fitness and PA indicators throughout weight loss interventions are not reported in the literature.

Hence, the primary purpose of the current study was to analyze the effects of a 16-month weight-loss intervention on thigh composition (SM area and quality, and AT area), CRF, and daily PA, in overweight and obese premenopausal women. The secondary objective was to study the associations between thigh composition changes and fitness and PA indicators alterations.

Experimental Methods

Participants Eligibility and Recruitment

Eligibility requirements were age ≥25 years, BMI ≥25 kg/m^2 and ≤39.9 kg/m^2, pre-menopausal, without diagnosis of hypertension, dyslipidemia, diabetes, thyroide alterations, or other diseases, and medication that could influence body composition. Women could not be pregnant and not willing to become pregnant in the next 2 years, and had to be willing to be randomly assigned to either an intervention or comparison group. Individuals were excluded if they were younger than 25 years old, presented a BMI lower than 25 kg/m^2 or higher than 39.9 kg/m^2, were post-menopausal, if they had any condition likely to increase risk from the intervention or to limit life span, any

condition that would likely affect the ability to conduct the intervention as designed, or if they were taking any medication or had any medical condition likely to confound the assessment of body composition.

Eligibility Visits

Eligible women, based on return of newspaper ads, poster information posted in health care facilities, a website, email messages on listservs, and announcement flyers, attended plenary sessions in which all the intervention procedures and study compliance were explained. If they agreed with the procedures, and were willing to participate, a demographic and clinical questionnaire was filled out, and they were invited to attend four screening visits during which eligibility was further assessed.

First baseline screening visit. An informed consent was signed according to the regulations of the Ethical Committee of the Faculty of Human Movement, Technical University of Lisbon. Body composition estimation was performed with anthropometry, bioelectric impedance spectroscopy, air displacement plethysmography, and DXA. Questionnaires assessing habitual PA, psychosocial and quality of life parameters, were administered during the first visit. A 4-day food record was assigned for return at the second visit.

Second baseline screening visit. The completion of the psychosocial questionnaires and of the 4-day food record was performed, and CRF was assessed.

Third baseline screening visit. Biochemical assessments and body composition by CT were performed.

Final eligibility. A clinical consultation with the intervention physician took place, in order to analyze the baseline results and decide, for each woman, if inclusion criteria were met, or if the subject should be excluded from the intervention.

Participants

Participants in the present study were 48 women, 27 to 49 years old, premenopausal, with overweight or obese conditions (25.2 to 38.2 kg/m^2). Participants received no financial compensation; instead they participated with the sole incentive of benefiting from the intervention.

Study Design

The intervention has a strong theoretical foundation, using an empirically-based cognitive-behavioral approach to promote the adoption and maintenance of a healthy lifestyle, with a strong emphasis on PA and nutrition [35]. The lifestyle intervention goals were to: 1) achieve and maintain a weight reduction between 5%-10% of initial body weight through healthy eating and PA; 2) achieve and maintain a level of PA of at least 200-300 min/week (equivalent to ~1500-2000 Kcal/week), through moderate-intensity activity; 3) achieve and maintain a reduction in total energy intake (300-500 Kcal/day less), with a reduction on fat intake (<25% of total daily intake), an increase in complex carbohydrates (CH; >45% of total CH daily intake), in fiber (>25 g/day), a daily protein intake of no more than 15% kcal/day, and the promotion of nutrient-dense, high-volume, low calorie foods such as fruits, vegetables and whole grains [35]. All the participants were submitted to a 16-week program, consisting of a 2-h weekly session aimed at promoting lifestyle changes through education and behavioral strategies concerning nutrition, PA and lifestyle management, as described by others [36]. After this 16-week program, participants were randomized to a comparison group, a monthly meetings group and a monthly meetings plus structured PA group. The design incorporates collection of outcome measures of all the participants at baseline and at 16-week, at 10-, 16-, and 22-month follow-ups (Figure 5.10).

Comparison Group

Each participant carried on with their life, away from the intervention team. Support from the intervention team could be asked by the women through telephone call or e-mail during the follow-up period.

Monthly Meetings Group

In this group participants benefited from a monthly meeting with the intervention group, during which goal setting, self-monitoring, stimulus control, problem solving, relapse prevention, social support, and motivational techniques, concerning nutrition, PA and lifestyle management were addressed.

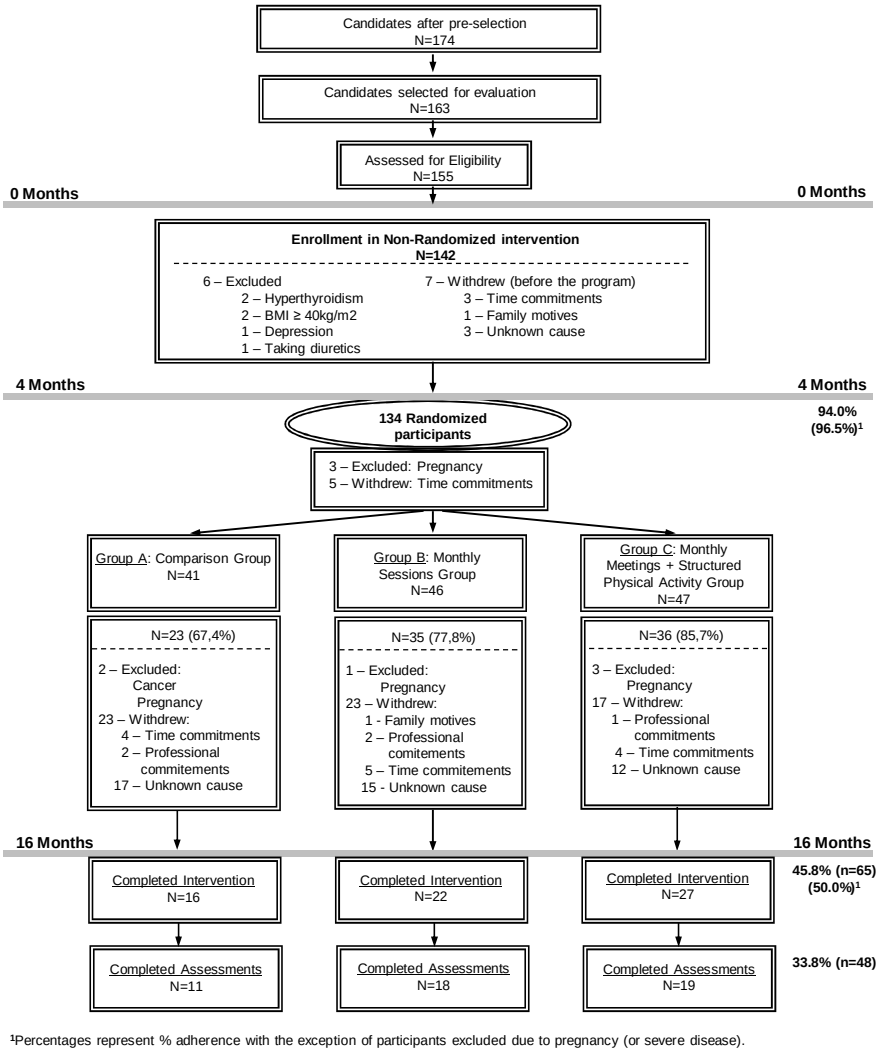
Monthly Meetings + Structured PA Group

Besides the monthly meetings, participants profited from 2 weekly sessions of structured exercise, performed between friday and sunday. The exercise program consisted of aerobic and strength training combined, to reach 300 kcal/session at the beginning of the program, progressing gradually to 400-500 kcal/session from 2-months on. Cardiovascular intensity was between 40%-50% heart rate reserve (HRR) when the intervention began and progressed to 55%-70% HRR. The modified scale of perceived exertion [37] was used by the participants aiming at a level between 4-8 on the scale. Strength training was incorporated, and the intensity was based on 1-RM assessment of upper and lower-body. Large muscle groups were included, and different muscle groups were used in each session and between sessions.

Monitoring the Intervention

Laboratory Measurements

At baseline and 16-months, biochemical analyzes and body composition assessment by CT were performed.



[1]Percentages represent % adherence with the exception of participants excluded due to pregnancy (or severe disease).

Figure 5.10. Consort diagram.

Subclinical Measurements

Body composition estimation (with anthropometry, bioelectric impedance spectroscopy, air displacement plethysmography, and DXA), and a maximal oxygen consumption test, were assessed at baseline and 16-months.

Other Measurements

PA, and psychosocial and quality of life parameters were estimated by questionnaires, and a 4-day food record was used to assess nutritional intake, also at baseline and 16-months.

Body Composition Measurements

Anthropometry

Body weight was measured with an electronic scale (BOD POD® Life Measurement Instruments, Concord, CA) to the nearest 0.1 kg, and height was obtained with the SECA scale stadiometer, measured twice to the nearest 0.5 cm. Accordingly with international organizations (NIH and WHO) guidelines for overweight and obesity, referred to as BMI over 24.9 kg/m^2 and 29.9 kg/m^2, respectively, BMI (calculated as kg/m^2) were used to define overweight and obese women among the study participants. Circumferences at the waist (1 cm above superior border of iliac crest), hip, midthigh and distal thigh [38] were assessed by a trained researcher. Based on 10 repetitions, the TEM and ICC were, respectively, 0.41 cm and 1.00 for waist circumference, 0.52 cm and 0.99 for hip circumference, 0.17 cm and 1.00 for midthigh circumference, and 0.37 cm and 0.97 for distal thigh circumference.

Dual-energy X-ray Absorptiometry

FFM and LST mass in the lower limbs were assessed by DXA (QDR-1500; Hologic, Waltham, MA, pencil beam mode, software version 7.2 enhanced whole body analysis), and the lower limbs region was defined using the standard analysis protocol for the whole body scan, described by the manufacturer. The same laboratory technician positioned the subjects, performed the scans and executed the analysis according to the operator's manual. Based on ten subjects, the coefficients of variation in our laboratory for FM and FFM were 2.9% and 1.7%, respectively.

Computed Tomography

Helical CT imaging (Siemens, Somaton plus) was performed using standard procedures as described by others [39], with scan parameters set at 120kVp for 1 s, 360 mA, 512 X 512 matrix, with a 48 cm field of view. The subject lied supine, with the arms extended above the head, and a 7-mm-thick cross sectional scan of both thighs was obtained in

each subject, between the inferior ischial tuberosity and the superior border of the patella, to assess thigh composition. Once accquired, the CT data were analyzed using commercially available software (Slice-O-Matic; Tomovision Inc., Montreal, Canada). Tissue segmentation was made using standard HU ranges: AT (-190 to –30 HU), SM (0 to +100 HU), normal density SM (+31 to +100 HU), and low density SM (0 to +30 HU) [40]. Because the skin is hardly identifiable in CT images, it was necessary to correct data, assuming that the skin had a thickness of 1 mm [41]. In both the right and left thighs, thigh AT tissue was further distinguished by manual tracing as: TTAT, TSAT, and TIAT, accordingly to Shen et al. classification [42], using thigh fascia lata to subdivide this AT compartments [39]. Bone marrow AT was excluded from this analysis. The number of pixels multiplied by the area of one pixel equals the tissue area for that CT slice [40].

The measurement reliability for thigh composition was performed with 3 months interval, analyzing the same images in 30 women. Only intraobserver error was calculated because all the measurements were performed by the same technician. The intraobserver CVs were 0.1% for thigh SM tissue, 0.4% for TSAT, and 2.5% for TIAT, as described by others [14].

Valid CT baseline measurements were performed on 142 women but from these 77 did not return for the second scan at 16-month, resulting in 65 subjects who had CT scans at both baseline and at the end of the intervention. From these 65 women, 17 were excluded from the present sample due to the absence of other assessments (CRF, PA, anthropometry, and/or DXA measurements), and consequently only 48 participants remained in the study sample.

Cardiorespiratory Fitness

To assess physical fitness at the beginning and at the end of the intervention, a breath-by-breath system measured airflow and volume continuously and, simultaneously, instantaneous expired CO_2 and O_2 concentrations were also determined. Respired gas volume, flow rates, and respiratory gas exchange were collected during exercise (MedGraphics® Corporation, utilizing BREEZEX Software). Maximal aerobic power (VO_2max), symptom-limited, was measured using an incremental protocol [43] on a motor-driven treadmill (Quinton Model 640 Treadmill Controller and Series 90TM Treadmills), with variable speed and grade, as described previously [44]. Due to the fact that this was a maximal test, reproducibility was not performed.

Physical Activity Questionnaire

PA was assessed with the International Physical Activity Questionnaire [45], an instrument with a mean rho for criterion validity of about 0.30. The short last seven days self-administered format was used, considering its reasonable measurement properties for monitoring population levels of PA among 18- to 65-yr-old adults in diverse settings [45], and in special populations such as obese subjects [46]. Information on several PA behaviors was provided, including walking, moderate-intensity and vigorous-intensity activities, and sitting. Computation of the total score for the short form requires summation of the duration (in minutes) and frequency (days) of walking, moderate-intensity and vigorous-intensity activities. Estimation of total PA per week was computed by weighting each type of activity by its energy requirements, defined in METs, to yield a score in MET–minutes [47]. The MET scores for each of the reported activities were derived using the Ainsworth et al. Compendium [48]. Reliability data were collected, and test-retest repeatability was performed within a two-week period.

Statistical Analysis

Normality was tested for all variables and for differences between baseline and 16-month assessments. Descriptive statistics of baseline and 16-month were computed as mean±SD, in the total sample and in each intervention group. Changes from 0 to 16-month (16-month measurement minus baseline measurement) were analyzed with the paired samples *t*-test, and with the Wilcoxon signed ranks test when normality was not verified. Differences between intervention groups, at the beginning and at the end of the intervention, were tested using ANOVA (time x intervention group). At 16-months the analysis of covariance (ANCOVA) was completed with PA variables entered as covariates.

Due to the number of subjects in the present sample (n=48), a bootstrap resampling analysis was performed to test for the specific indirect (or mediated) effects [49]. The mediation models used the intervention group as the independent variable, while changes in thigh composition variables (SM areas and quality, and AT areas) were tested as dependent variables. Age, BMI, and PA changes were tested as mediators. An SPSS macro provided by Preacher and Hayes (2007), with multiple linear regression analysis was used to analyze the causal steps criteria for mediation forwarded by others [50]. A resampling procedure (5000 bootstrap samples), via the Bias Corrected and Accelerated (BCa) estimates and 95% confidence intervals to present the indirect effects significance were performed. If the BCa 95% interval confidence did not included zero, it can be concluded that there was a significant indirect effect (at alpha=0.05) [49].

Multiple linear regression models were used to analyze the associations between changes in thigh composition variables, and alterations in other body composition variables, controlling for age and BMI. Statistical analysis was performed using the

Statistical Package for Social Sciences (SPSS inc., 17.0 version, Chicago, IL), and MedCalc Statistical Software (MedCalc Software, Mariakerke, Belgium). Statistical significance was set as $P<0.05$.

Results

Mean and SD, of all the studied variables, at the beginning (0-month) and at the end of the intervention (16-month), are presented in Table 5.8. None of the baseline measurements differed significantly ($P>0.05$) by intervention group (Table 5.8). On average, participants were obese, with a mean BMI of 30.0±3.0 kg/m^2. The waist circumference was large. Absolute FM was high, both in total body, and in the lower limbs, almost ranging LST results. CT data revealed that TTAT tissue area was greater than SM tissue area. Normal density SM area was the major component of thigh SM tissue, while TSAT was the greatest contributor to thigh AT. Low density SM and TIAT areas were the smaller components of thigh SM and AT, respectively. SM density was low indicating a high lipid infiltration. The low CRF level was in agreement with time spent in PA during the week.

In the total sample, all the body composition measurements decreased from baseline ($P<0.001$ for all), with the exception of low density SM and TIAT areas that increased during the intervention ($P<0.001$). Low density SM area increased and this increment occurred simultaneously with a reduction in SM quality reflected by decreased HU. No significant changes were observed during the intervention for SM and normal density SM areas and quality, as well as for lower limbs LST ($P>0.05$ for all).

Table 5.8. Baseline and 16-month characteristics, in the total sample and by intervention group: comparison group (Comp. Group), monthly meetings group (M.M. Group) and monthly meetings+structured PA group (M.M.+SPA Group). P-value of the differences from baseline to 16-months (t-test and Wilcoxon signed ranks test).

	Overall		**Comp. Group**		**M.M. Group**		**M.M.+SPA Group**	
	Baseline X±SD	**Post-16m X±SD**	**Baseline X±SD**	**Post- 16m X±SD**	**Baseline X±SD**	**Post-16m X±SD**	**Baseline X±SD**	**Post-16m X±SD**
N	48	48	11	11	18	18	19	19
Age (years)	39.2±5.7	--	38.5±4.6	--	38.1±5.8	--	40.8±6.1	--
Weight (kg)	76.9±9.2	71.8±9.0***	76.1±10.0	72.4±7.9**	75.9±6.9	70.1±7.7***	78.3±10.8	73.1±10.7**
Stature (m)	1.6±0.05	--	1.6±0.05	--	1.6±0.04	--	1.6±0.05	--
BMI (kg/m^2)	30.0±3.0	28.0±3.0***	30.0±3.3	28.6±2.9**	29.2±2.4	27.0±2.8***	30.7±3.3	28.7±3.7**
Anthropometry								
Waist circumference (cm)	90.1±6.9	85.0±8.3***	89.8±5.7	85.8±5.7***	88.8±6.1	82.9±7.9***	91.4±8.2	86.5±9.8**
Hip circumference (cm)	111.5±6.7	107.7±7.3***	109.9±7.3	107.7±7.0	110.7±5.5	106.2±6.5***	113.2±7.2	109.0±8.1**
Midthigh circumference (cm)	57.6±3.9	54.9±4.3***	57.3±4.7	55.0±4.6**	57.0±2.8	54.0±3.0***	58.3±4.5	55.7±5.1**
Distal thigh circ. (cm)	39.7±2.8	38.7±2.9***	40.0±3.7	39.2±3.6*	39.4±1.5	38.1±1.7***	39.9±3.2	39.0±3.3§
DXA								
Lower limbs FM (kg)	13.3±3.2	11.6±2.9$^{¥¥¥}$	13.4±4.0	11.9±3.6$^{¥¥}$	12.7±2.4	11.0±2.2$^{¥¥¥}$	13.9±3.4	11.9±3.2$^{¥¥¥}$
Lower limbs LST (kg)	12.2±1.6	12.3±1.6	11.7±1.7	11.9±1.8	12.3±1.8	12.3±1.6	12.3±1.5	12.5±1.5
Computed Tomography								
SM area (cm^2)	229.4±28.0	229.7±29.4	217.3±22.1	223.3±29.5	234.0±34.6	231.3±34.4	232.0±23.1	231.8±24.8
SM quality (HU)	44.1±2.5	44.5±3.3	43.5±3.0	43.7±3.9	44.7±2.8	45.2±2.6	43.8±1.9	44.2±3.5
Normal density SM area (cm^2)	185.4±24.1	187.0±27.5	174.8±20.6	178.5±29.5	190.0±28.8	191.1±31.5	187.1±20.1	188.0±22.0
Normal density SM quality (HU)	51.6±1.4	52.2±2.2	51.4±1.5	52.1±2.7	52.0±1.7	52.6±1.9	51.5±1.2	51.8±2.3
Low density SM area (cm^2)	31.7±7.5	43.0±10.8***	30.9±9.4	45.0±11.0***	31.0±7.7	40.6±8.8***	32.7±6.3	44.1±12.4***
Low density SM quality (HU)	18.1±0.4	11.0±1.0***	17.9±0.4	10.8±0.8***	18.1±0.4	11.0±1.0***	18.2±0.4	11.2±1.1***
TTAT area (cm^2)	271.2±72.2	248.4±68.8***	270.8±93.9	258.7±84.2	249.1±49.3	226.7±48.7**	292.3±73.9	263.0±73.5**
TSAT area (cm^2)	261.7±71.1	240.0±67.3***	259.7±92.4	248.5±83.1	240.4±48.8	218.9±48.3**	283.0±72.6	255.1±71.2**
TIAT area (cm^2)	3.7±2.1	8.6±3.9***	3.1±1.2	10.5±2.9***	3.8±2.5	8.0±4.2***	3.9±2.1	8.1±3.8**
Cardiorespiratory Fitness								
VO_2max (ml/kg/min)	24.7±4.2	28.5±6.1$^{¥¥¥}$	23.2±2.23	27.3±5.0$^{¥¥}$	26.1±4.2	29.8±5.2$^{¥¥¥}$	24.1±4.8	28.1±7.4$^{¥¥}$
Physical Activity								
TtPA (min/week)	168.1±217.1	326.8±240.8**	137.3±147.9	323.6±335.8	184.7±262.7	314.2±246.5	170.3±211.9	340.5±176.0**
MVPA (min/week)	82.1±134.8	169.6±187.9$^{¥¥}$	49.1±85.1	175.9±246.2	98.9±146.6	156.9±189.1	85.3±148.7	177.9±156.7$^{¥¥}$

BMI, body mass index; Circ., circumference; FM, fat mass; HU, Hounsfield Units; LST, lean soft tissue; MVPA, moderate to vigorous physical activity; PA, physical activity; SD, standard deviation; SM, skeletal muscle; TIAT, thigh intermuscular adipose tissue; TSAT, thigh subcutaneous adipose tissue; TTAT, total thigh adipose tissue; TtPA, total physical activity; VO_2max, maximal oxygen consumption; X, mean.

*t-test; *P<0.05; **P≤0.01; ***P≤0.001. $^{\S}P$=0.053. $^{¥}$Wilcoxon signed ranks test; $^{¥}P$<0.05; $^{¥¥}P$<0.01; $^{¥¥¥}P$≤0.001.

Maximal oxygen consumption presented a significant increase ($P<0.001$), indicating a 15.4% improvement. An increase in PA performed during the week ($P<0.001$) and in time spent in moderate-to-vigorous PA (MVPA; $P<0.01$) was also observed during the same time period. These results are similar to those observed when each intervention group was analyzed separately. Exceptions to this situation occurred with the hip circumference, TTAT and TSAT areas, that revealed no differences ($P>0.05$) from baseline to 16-month in the comparison group, and also with total PA and MVPA, that were only significantly higher ($P<0.01$) in the monthly meetings + structured PA group (Table 5.8).

Despite the intervention design, and the changes observed from baseline to 16-month, once again, no differences ($P>0.05$) were observed between the three groups at the end of the intervention, even when PA was controlled. These results were confirmed by the bootstrap analysis which showed that the total and direct effects of the intervention group on the thigh composition variables were not significant ($P>0.05$), indicating that the intervention group did not influenced changes in the dependent variables.

Regression models of the changes of thigh composition variables and the other variables are presented in Table 5.9. These results revealed that the increase of low density SM area was positively associated with the modifications of weight, BMI and all the anthropometric variables: waist, hip, midthigh circumference, and distal thigh circumference ($P\leq0.001$ for all). Positive associations were also found between the changes of low density SM area and alterations of lower limbs FM ($P\leq0.01$). The increase of low density SM area was inversely associated with changes in maximal oxygen consumption, and this association remained after adjustment for total and MVPA alterations ($P\leq0.01$ for all). The significant decrease of low density SM quality presented a direct association with the changes in weight, BMI, distal thigh

circumference, and lower limbs LST (P<0.05 for all), and was negatively related to CRF modifications (P<0.05), although this association became marginally significant after controlling for modifications of PA (P=0.052 for both total PA and MVPA).

TTAT and TSAT areas decreases during the 16 months were positively associated with the alterations of weight, BMI, anthropometric variables, and lower limbs FM change, and negatively related with maximal aerobic capacity, even after adjusting for changes in PA variables (P≤0.001 for all). The increase observed for TIAT, from the beginning to the end of the intervention, was directly associated with the modifications of weight, BMI (P≤0.01 for both), waist, hip (P≤0.001 for both), midthigh circumference (P<0.05), and lower limbs FM (P≤0.001). These results can be observed in Table 5.9.

Discussion

To our knowledge, this was the first study to show that a 16-month intervention program aimed to promote the adoption and maintenance of a healthy lifestyle, with a strong emphasis on PA and nutrition, produced significant and beneficial changes in CT-determined low density thigh SM area and quality, TTAT and TSAT, as well as in CRF, PA, anthropometric, and DXA body composition variables, in previously sedentary, overweight and obese premenopausal women. An increase was observed in TIAT. These results indicate that in these women, a behaviourally-based intervention can significantly impact selected body composition changes detected by CT along with the increase of fitness and PA.

Table 5.9. Linear regression analysis (unstandardized beta) for the alterations in the selected dependent variables and changes (0m-16m) in all the others variables.

	Δ SM area	Δ SM HU	Δ NDens. SM area	Δ NDens. SM HU	Δ LDens. SM area	Δ LDens. SM HU	Δ TTAT area	Δ TSAT area	Δ TIAT area
Age (years)	-0.458	-0.112	-0.786**	-0.052	0.291	-0.004	1.057	0.867	0.184
Δ Weight (kg)	0.892**	-0.205*	0.014	-0.087	0.896***	0.051*	6.057***	5.832***	0.300**
Δ BMI (kg/m^2)	2.254**	-0.522*	0.012	-0.217	2.289***	0.132*	15.611***	15.012***	0.764**
Δ Waist (cm)	0.928**	-0.217*	-0.113	-0.084	1.099***	0.048	6.303***	6.057***	0.452***
Δ Hip (cm)	0.949	-0.171	0.039	-0.040	0.995***	0.046	6.831***	6.594***	0.428***
Δ Midthigh circumference (cm)	1.441*	-0.431*	-0.282	-0.218	1.833***	0.094	10.984***	10.629***	0.658**
Δ Distal thigh circ. (cm)	2.584*	-0.663*	-0.072	-0.321	2.697***	0.175*	14.285***	13.454***	0.669
Δ Lower limbs FM_{DXA} (kg)	2.057*	-0.580*	-0.354	-0.221	2.729***	0.120	17.972***	17.198***	1.095***
Δ Lower limbs LST_{DXA} (kg)	10.786***	-0.866	7.390*	-0.705	4.851*	0.563*	11.858	12.611	1.475
Δ VO_2max (ml/kg/min)	-0.072	0.134	0.470	0.058	-0.534**	-0.045*	-3.049***	-2.924***	-0.159
Δ VO_2max (ml/kg/min) Adj. ΔTtPA	0.011	0.132	0.440	0.058	-0.532**	-0.045	-3.023***	-2.902***	-0.153
Δ VO_2max (ml/kg/min)Adj. ΔMVPA	0.020*	0.133	0.362	0.060	-0.532**	-0.045	-2.899***	-2.791***	-0.146
Δ Total PA (min/week)	0.010	0.001	0.008	0.000	-0.002	0.000	-0.012	-0.011	-0.002
Δ Total PA Adj. ΔVO2	0.010	0.000	0.008	0.000	-0.001	0.000	-0.007	-0.006	-0.002
Δ MVPA (min/week)	0.019*	0.001	0.021**	1.17E-005	-0.003	4.931E-5	-0.040	-0.036	-0.003
Δ MVPA Adj. ΔVO2	0.019*	3.27E-005	0.019*	0.000	0.000	-0.045	-0.026	-0.023	-0.002

Adj., adjusted for; BMI, body mass index; Circ., circumference; FM, fat mass; HU, Hounsfield Units; LDens., low density; LST, lean soft tissue; MVPA, moderate to vigorous physical activity; NDens., normal density; PA, physical activity; SM, skeletal muscle; TIAT, thigh intermuscular adipose tissue; TSAT, thigh subcutaneous adipose tissue; TTAT, total thigh adipose tissue; TtPA, total physical activity; VO_2max, maximal oxygen consumption; Δ, changes from baseline to 16-months.
*P<0.05; **P≤0.01; ***P≤0.001.

An increment was verified in the low density SM area, which is the component of SM with higher lipid infiltration, along with a decrease in the respective attenuation coefficients. These findings are different from the ones found in the literature [33, 34] during weight loss interventions with aerobic exercise, in older men and women, which showed a decrease of low density SM area. At the individual level, current results indicate that women who presented smaller low density SM area increments revealed greater low density SM lipid infiltration concomitant with improvements in CRF, during the 16 months. Conversely, the participants with greater low density SM area increments showed lesser lipid infiltration of this SM compartment along with minor gains of maximal oxygen consumption. Although CT is not capable of distinguishing extramyocellular (EMCL) and intramyocellular lipid content (IMCL), SM attenuation coefficients determined by CT reflect both IMCL and EMCL content [51]. Hence, even though this is the first study to examine the effects of a weight loss intervention on changes in thigh low density SM specifically in overweight and obese premenopausal women, these results suggest the occurrence of the training paradox [52]. Accordingly, an increase in IMCL content has been observed, both, in endurance training athletes and in the insulin resistance state. However, in the first condition IMCL is a readily available energy source, as long as the oxidation capacity is also increased as a result of endurance training, while in the second condition IMCL is an outcome of high fat availability and low fat oxidation [53]. Also, evidences have been found indicating that probably fat oxidative capacity is more important than IMCL content in determining insulin sensitivity [52, 54], including in older adults [55], and that the ratio between intramuscular triacylglycerol content and SM oxidative capacity represents a more accurate marker of insulin resistance [56]. In addition, it was found [55] that aerobic exercise training induced as well a shift in muscle fiber type toward more oxidative type

I muscle fibers, which have been shown to have higher IMCL content [57]. Therefore, in the present study, the increase in SM lipid infiltration in women with greater increments in CRF (an indicator of greater fat oxidative capacity) could represent an adaptive response allowing IMCLs to serve as an energy source for PA. Conversely, those participants with lower improvements in CRF, and lower increases in SM lipid infiltration, probably present an impaired capacity of SM mitochondria, that could have detrimental effects on insulin sensitivity if IMCLs are not being used as an energy substrate [52, 58, 59]. The greater increases in low density SM area were verified in women who, in addition, showed a higher decrease of weight, BMI, waist, hip and thigh circumferences, as well as in lower limbs FM. As these body composition indicators reflect a decrease in the whole body and lower limbs FM, they emphasize the greater oxidative capacity previously referred through the increase in CRF. Current results are reinforced by those of others [60], accordingly to whom exercise combined with weight loss enhances postabsorptive fat oxidation, which appears to be a key aspect of the improvement in insulin sensitivity in obesity. Besides, in the present findings, the associations between low density SM area and CRF alterations occurred independently from total and MVPA changes, emphasizing the contribution of oxidation capacity regarding that thigh SM component. Therefore, the current study seems to extend previous findings indicating that the increase in IMCLs might not be limited to intensive training (as in athletes; [54]) nor to sedentary young men [61], but it could also be achieved through increased habitual PA in overweight and obese premenopausal women.

Although overall changes are noteworthy, no significant modifications were observed between intervention groups at 16-month. These findings were probably due to the study design, accordingly to which all participants were first submitted to a 16-week

lifestyle promotion program prior to randomization to one of the three intervention groups: comparison group, monthly meetings group or monthly meetings+structured PA group. This initial lifestyle program might have produced a contamination effect on the comparison group that, although has lost contact with the intervention staff in the subsequent 12 months of the study, had already benefited from a cognitive-behavioural approach to promote the adoption of a healthy lifestyle that led to behavioural changes, concerning PA and nutrition (data not shown).

Despite the fact that no changes were observed between groups at 16-months, significant alterations were found in each intervention group from baseline to the end of the study. Thigh SM and normal density SM areas, as well as their quality, were not significantly altered during the intervention, and these findings were reinforced by DXA results which revealed no changes in the lower limbs LST. Two factors may have contributed to these results: although CRF was significantly increased, the maximal aerobic capacity of these women remained low (change from 10^{th} percentile at baseline to 10^{th} percentile < CRF < 20^{th} percentile at 16-months; [62]), therefore the PA dose probably was not enough to induce total thigh SM and normal density SM changes, which may require more intense and greater volume activities in order to be accomplished, as observed by others authors [30, 31, 63]. These authors, in studies evolving subjects with different health conditions, observed improvements in SM with structured exercise, performed 3-5 d/week, 1-2 h/session, of at least moderate intensity. Although this PA volume was not higher from the one found in the present study, current results are self-reported and it is possible that over-reporting has occurred, as mentioned by other studies [64], influencing PA data. Besides PA dose, another contributor to the present results relies on the fact that all the women lost weight and, as reported by others [65-67], the weight loss occurs simultaneously with AT reduction,

and also with SM decrease, as verified in different age groups and weight conditions. Therefore, the combination of weight loss with the low CRF of the participants may have not allowed total SM increase to take place.

The decrease of TTAT and TSAT areas were only observed in the monthly meetings and in the monthly meetings+structured PA groups. The comparison group did not revealed significant changes in the TTAT and TSAT compartments. Women that lost more TTAT and TSAT were those who presented a greater increase in CRF, independently of their PA modifications. As referred by others [68], the higher increase in maximal aerobic capacity allowed greater AT mobilization ability for energy production, promoting AT decrease. CT alterations concerning the decrease of TTAT and TSAT were also verified simultaneously with the reduction of weight, BMI, waist, midthigh circumference and lower limbs FM in all the intervention groups. Hip circumference decreased only in the two monthly session groups, while no significant modifications were observed in the comparison group, highlighting the differences of the changes observed between the comparison group and the other intervention groups.

The literature [11] refers that TIAT determined by CT presents an enlargement with increasing BMI (1 cm^2 versus 5 cm^2 in lean and obese glucose tolerant individuals, respectively), and in T2DM (9 cm^2). Others authors [69] observed that a 1-kg increment in body FM, in obesity, was associated with a 0.5-0.1 cm^2 increase in TIAT. More recently [28], it was observed that, in older persons, PA combining aerobic, strength, flexibility, and balance training, prevented TIAT increase when compared to controls. These findings are contradictory to our results that demonstrate an increase in TIAT, which more than doubles from baseline to 16-month, in the three intervention groups, despite weight loss, approaching the literature values mentioned for T2DM condition. Furthermore, the increment was greater in participants who lost more weight and BMI,

and who showed higher decrease of waist, hip and medial thigh circumferences, in addition to a major reduction of lower limbs FM. In what regards CRF, a significant increment was observed in all the three groups. PA changes throughout the intervention were only significant in the monthly meetings+structured PA group, but a wide variation was observed in total PA as in MVPA.

Strengths of the present study include the body composition assessment by CT, allowing for thigh composition changes monitoring throughout the intervention. Imaging methods are considered the reference for the assessment of body composition, but due to the high cost and the radiation exposure of the CT, most of the research refers only to CT studies performed with a single slice at the midthigh, as in the current study. Another strength of this research was the intervention duration which lasted 16 months.

The major limitation of the current research was the use of a self-administered questionnaire to assess PA. Although questionnaires are low cost, ready available, and easy to administer instruments, to date there is no formal consensus on a 'correct' method for defining or describing levels of PA based on self–report population surveys [47]. The IPAQ was developed in an attempt to standardize the assessment of health-related PA based on self–report population surveys [45]. Research indicates that the short, last 7-days, version of the IPAQ instrument significantly overestimated self-reported time spent in PA, in adults [64], while other results, indicate that habitual PA report in obese subjects with the IPAQ warrants further evaluation against objective assessment methods [46]. Another limitation was the sample size. However, this limitation was somewhat offset by the bootstrap resampling analysis [49].

Conclusions

In brief, a 16-month weight-loss intervention positively impacted low density SM thigh

tissue area, along with a decrease in low density SM quality and increases in CRF, and daily PA, suggesting the occurrence of the athlete's paradox in overweight and obese premenopausal women. Significant improvements were found in each intervention group from baseline to the end of the study concerning body composition, maximal aerobic capacity and PA, despite no significant modifications were observed between intervention groups at 16-month.

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CHAPTER 6

General Discussion

"At least once in a lifetime it is convenient to put everything to discussion."

Descartes

Main Research Findings

The general introduction (**Chapter 1**) identified body composition components, namely SM and AT, quantified by imaging methods state of the art, focusing on thigh composition. The effect of some biological factors, such as aging, gender, and several health conditions, on thigh SM and AT were analyzed. Special emphasis was given to computed tomography (CT), dual-energy X-ray absorptiometry and anthropometry due to the fact that these methods were used in the development of the present Thesis. The associations between thigh composition and fitness and PA indicators, in obesity and weight loss, were also reviewed. This Thesis aimed to analyze thigh composition in overweight and obese premenopausal women, and its associations with CRF and PA, at baseline and after a 16-months weight loss intervention. **Chapters 3, 4, and 5** present the original research contributions. The three studies collected in this dissertation fall into the areas of body composition methodology (S**tudy I in chapter 3**) and body composition variation (**Studies II and III in chapters 4 and 5, respectively**).

The multidisciplinary interests and the need for methods to estimate thigh components, reflect its importance and applicability in different settings, such as physiology, clinical medicine, nutrition, and evaluation of treatments and interventions [1-4]. Beside, thigh composition vary widely in SM and AT content in diverse health conditions. So, the area of body composition methodology was addressed by means of investigating, in a cross-sectional design, the evaluation and calibration of existing techniques to be used in thigh composition estimation. Prediction equations for the different thigh components (SM tissue, SM quality, TTAT, TSAT e IMAT) specific to overweight and obese premenopausal women have never been developed. Two widely used body composition methods, DXA and anthropometry, were employed to assess thigh composition predictors with CT as the reference. This procedure allows to surpass the high cost,

radiation exposure and time required for image analysis by CT assessments. The SM tissue mass and quality developed prediction models revealed no significant differences when compared with the reference method. In what concerns TSAT, an underestimation was observed, while overestimations were verified for TTAT and TIAT. The new models may be particularly useful in the clinical practice and sports settings where body composition may be critical and need to be followed longitudinally, such as in wasting illnesses.

In the area of body composition variation, the specificity of thigh composition in previously sedentary, overweight and obese premenopausal women, and its associations with objectively measured PA and CRF was investigated. The low values obtained for thigh SM density showed the lipid infiltration of SM, indicating a poor SM quality in the participants. These results are in agreement with earlier investigations that referred lower attenuation coefficients in obese subjects SM when compared to normalweight condition [5-7]. Women who performed more low-intensity PA revealed lower TSAT and TIAT, while the time spent in sedentary behavior was associated with higher AT in this body region. Those women with greater CRF showed higher thigh SM mass, as reported previously but in obese older women [8]. A Z score for thigh composition showed a graded increase across CRF tertiles, representing higher thigh SM mass and quality, along with lower AT accumulation, in women with greater oxygen consumption. These results suggest that CRF level is associated with different thigh composition phenotypes.

With the worldwide increase of obesity epidemic and its associated comorbidities, the treatment of this health condition is assuming greater relevance. The implementation of a 16-months behaviourelly-based intervention, with particular emphasis on PA and

nutrition, produced beneficial changes in CT-determined thigh composition alongside with the increase of fitness and PA. The increment of low density SM area in conjunction with the decrease in low density SM quality and the increase of CRF, and daily PA, suggest the occurrence of the training paradox in overweight and obese premenopausal women. Accordingly, the increment in SM lipid infiltration in women with greater improvements in CRF, which is an indicator of fat oxidative capacity [9, 10], could represent an adaptive response allowing IMCL to serve as an energy source for PA, as observed in earlier investigations [11, 12]. Therefore, current research seems to extend previous findings [13, 14] indicating that the increase in IMCL might not be limited to intensive training but could also be achieved through increased habitual PA in overweight and obese premenopausal women. Overall changes from baseline are noteworthy regarding either the decrement in FM and AT indicators (weight, BMI, body circumferences, lower limbs FM, TTAT and TSAT areas), as well as CRF and PA increases.

Interactions Between the Two Body Composition Research Areas

In the general introduction the three distinct areas of body composition research were indicated. These areas are body composition rules and models, body composition methodology and body composition variation. Due to the fact that the current Thesis involves the body composition methodology and variation, the interrelations between these two research areas are discussed. The possible pathways linking these two areas are described in Figure 6.11.

The study of body composition methodology promotes the development of body composition variation evidences. More precisely all descriptive (or type I) body composition methods provide the necessary techniques to characterize and follow-up

individual and population *in vivo* body composition variation at different settings, allowing the assessment of the major body components of the five levels. Conversely, the knowledge of body composition particularities, accordingly to biological and pathological specificities, indicate strengths and limitations of body composition assessment techniques, highlighting, also, the improvements needed for *in vivo* body composition measurement. This means that the knowledge of body composition variation is required in order to allow the development of more accurate methods. As a result, body composition variation knowledge is the basis for the progress of more accurate *in vivo* methods.

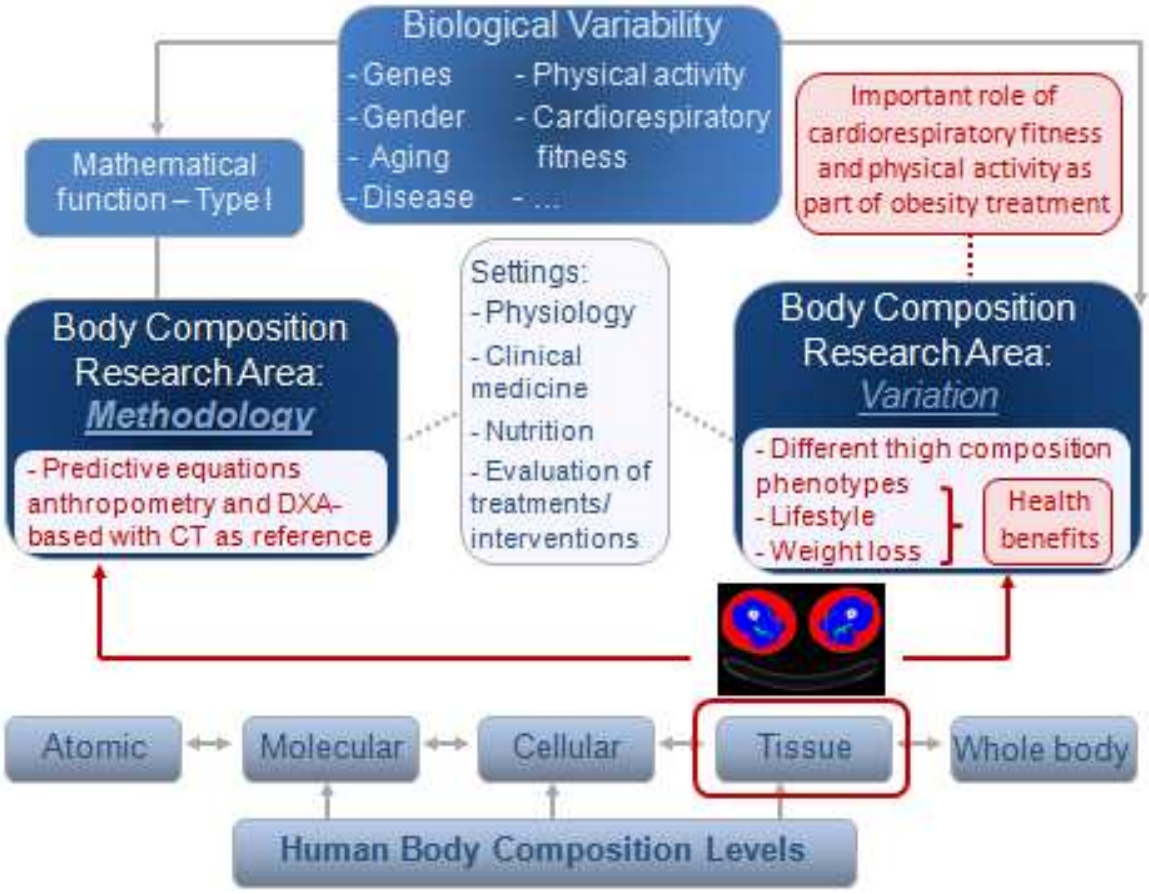

Figure 6.11. Pathways linking body composition methodology and body composition variation areas.

In what concerns the innovative input of the current Thesis to this model, an example of these interrelationships rely on the ability of different methods, namely, CT, DXA and anthropometry, to detect changes in thigh components at, respectively, the tissue-organ, molecular and whole body levels of body composition analysis, which can be mediated by PA and CRF, in overweight and obese premenopausal women that underwent a weight loss program. Another original contribution refers to the fact that, besides the biological influences of genes, gender, aging, and disease (namely, overweight and obesity conditions), CRF and PA also added to the variation observed in thigh composition of the study participants. The associations established between those biological variables and thigh components highlighted diverse thigh composition phenotypes, adding to the scientific research area of body composition variation. Lifestyle habits, specifically PA and nutrition, play an important role, affecting biological variability and inducing body composition changes in the thigh region, even at the setting of a weight loss intervention.

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CHAPTER 7

General Conclusion

"What is important is to keep learning, to enjoy challenge, and to tolerate ambiguity. In the end there are no certain answers."

Martina Horner

Summary of the Main Research Findings

The main contributions of the current Thesis are drawn as follows:

- In **Study I** (**see chapter 3**), thigh composition prediction equations were developed and validated in overweight and obese premenopausal women. Table 7.10, presents the developed models for SM tissue, SM quality, TTAT, TSAT and IMAT.

Table 7.10. The main contribution from **chapter 3**.

Predictive Equations	**R^2**	**SEE**
SMHU (HU)=61.631–(0.102 x Age)-(0.203x FM)-(0.318x$DistalT_{Circ}$)+[6.24E-008x(Hip_{Circ}xPrT_{Circ})^2]	0.425	1.863
SM (kg)=1.502+(0.462x LST_{Ll})-(0.030x$DistalT_{Circ}$)	0.840	0.309
TTAT (kg)=-2.294+(0.436xFM_{Ll})+(0.027xFM)+(0.001x Hip_{Circ}xPrT_{Circ})	0.941	0.493
TSAT (kg)=-1.881+(0.479x FM_{Ll})+[0.001x(PrT_{Circ})^2]	0.943	0.468
TIAT (kg)=-1.388+(0.039x%FM)-(0.009xHip_{Circ})-(0.036xLST)	0.559	0.143

Abbreviations: $DistalT_{Circ}$, distal thigh circumference; FM, total body fat mass estimated by DXA; FM_{Ll}, lower limbs fat mass estimated by DXA; Hip_{Circ}xPrT_{Circ}, hip circumference multiplied by proximal thigh circumference; LST, total body lean soft tissue mass; LST_{Ll}, lean soft tissue mass of the lower limbs; PrT_{Circ}, proximal thigh circumference; R^2, coefficient of determination using the PRESS method; SEE, standard error of measurement using the PRESS method; %FM, relative fat mass.

- **Study II** (**see chapter 4**), suggest that in previously sedentary overweight and obese premenopausal women, CRF is positively associated with thigh SM quantity, represented by SM mass estimated by CT scans, while TSAT is negatively influenced by low-intensity PA. In these women, maximal oxygen consumption level is associated with different thigh composition phenotypes. More precisely:

  - Women with higher levels of CRF presented greater thigh SM area, independently from their PA level.
  - Participants who performed more low intensity PA showed lower TSAT.

- The increment of VO_2max (mL/kg/min) was associated with a healthier thigh composition, derived from a Z-score combining thigh SM mass, thigh SM quality, TSAT and TIAT.

- In **Study III** (**see chapter 5**), a 16-month weight-loss intervention positively impacted thigh low density SM tissue area, along with a decrease in low density SM quality and increases in CRF, and daily PA, suggesting the occurrence of the training paradox in overweight and obese premenopausal women. Significant and beneficial improvements were observed in each intervention group, from baseline to the end of the study, regarding body composition, maximal aerobic capacity and PA, although no differences were found between groups. In particular:

- Greater increments in CRF together with the enlargement in SM lipid content, achieved through the increase of habitual PA, could represent an adaptive response indicating the role of IMCL as an energy source for PA, thus implying the possibility of the athlete's paradox effect.
- The intervention design did not produce different results among the studied groups.
- Favourable changes were observed from 0-month to 16-month, more precisely concerning CT-determined thigh low density SM area and quality (+11.3 cm^2 and -7.1 HU, respectively), TTAT (-22.8 cm^2), TSAT (-21.7 cm^2), simultaneously with the decrease of all anthropometric indicators (with the exception of stature), and DXA estimated FM in the lower limbs, along with improvements in CRF (+3.8 ml/kg/min) and daily PA (+158.7 min/week for total PA and +87.5 min/week for MVPA).

Added Value of the Current Thesis

This Thesis was designed in the relevant context of obesity treatment with the purpose of providing scientific knowledge on thigh composition characteristics of overweight and obese premenopausal women, through a comprehensive analysis of body composition methods and alterations research areas. Evidence-based findings revealed associations between thigh SM and quality, as well as AT compartments, with several health risk conditions, particularly in overweight and obese individuals highlighting the relevance of this research aim. Alongside, the associations of thigh components with maximal oxygen consumption and PA, recognized by the literature as important health contributors, were also addressed cross-sectionally, as well as longitudinally, during a weight loss intervention.

Scientific research in this field of knowledge revealed the need for easy applicable methods to estimate thigh components, namely, SM and AT, at different settings, and with diverse weight and health conditions. Therefore, thigh composition predictive equations based on an imaging reference method (CT), and using DXA and anthropometric indicators, specifically for overweight and obese premenopausal women, were not available until now. Thus, the current Thesis findings represent an original contribution to help fulfill that gap, enhancing thigh SM and AT assessment to be used this population. The developed procedures also allow to surpass the high cost, radiation exposure and time consumed for image analysis, required by CT.

In the present work, a greater lipid infiltration of SM in obese subjects was found, which is in accordance with the literature. Besides that, this Thesis added evidence concerning the relevance of CRF and PA associations with thigh components, namely: women with a higher CRF presented greater SM mass, and TSAT was lesser in

participants who performed more low-intensity PA. The importance of CRF status was highlighted in the present work, showing that overweight and obese premenopausal women with higher CRF revealed a healthier thigh composition phenotype, with greater SM mass and SM quality, concurrently with lower TSAT and TIAT.

Another original contribution of the current Thesis, and also a reinforcement of the relevance of CRF and PA associations with thigh composition, in overweight and obese premenopausal women, was found in the analysis of a 16-month weight-loss intervention. More precisely, the present work suggests that the increase in CRF, and daily PA, together with the increment in SM lipid content, could be the expression of an adaptive response, in which IMCL act as an energy source for PA, as observed in the literature but for other populations, and with exercise training regimens instead of PA. These adaptations occurred simultaneously with the decrease of the anthropometric indicators, and lower limbs FM estimated by DXA.

In summary, predictive equations for thigh components, based in DXA and anthropometry, are now available for overweight and obese premenopausal women, to be used at different settings and health conditions. CRF and PA should be targeted in the treatment of overweight and obese premenopausal women, in order to induce favorable adaptive changes in thigh composition, which are accompanied by weight loss and improvements in whole body composition. It is important to highlight that the health-associated benefits resulting from the increment of CRF and PA surpass their influence on body composition and weight control, playing an important key-role by improving biomarkers and related health status. Thus, an active lifestyle and more important, CRF-related improvement through moderate and vigorous PA can induce healthier phenotypes in obese individuals.