University of São Paulo

School of Public Health

Associations of birth weight with dual-energy x-ray absorptiometry-determined body composition, bone densitometry and cardiometabolic risk profile in young women from the Nutritionists' Health Study

Angélica Marques Martins Valente

Doctoral Thesis submitted to the Graduate Program in Epidemiology

Concentration area: Epidemiology

Counselor: Professor Sandra Roberta G. Ferreira Vivolo

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4. Body composition	5. Visceral adipose tissue	6. Osteosarcopenia

DEDICATION

Esta tese é dedicada:

*Às **participantes** que, generosamente, contribuíram com o estudo.*

A cada uma dediquei o meu melhor, sempre fundamentada no respeito e ética.

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Onde houver ofensa, que eu leve o perdão.
Onde houver discórdia, que eu leve a união.
Onde houver dúvidas, que eu leve a fé.
Onde houver erro, que eu leve a verdade.
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compreender, que ser compreendido;
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ABSTRACT

Valente, AMM. Associations of birth weight with dual-energy x-ray absorptiometry-determined body composition, bone densitometry and cardiometabolic risk profile in young women from the Nutritionists' Health Study [thesis]. São Paulo: School of Public Health, University of São Paulo; 2019.

Background: Visceral adiposity is a risk factor for cardiometabolic diseases and dual-energy x-ray absorptiometry (DXA) represents precise method for measuring visceral adipose tissue (VAT), muscle and bone compartments. The musculoskeletal system deteriorates with aging and may result in osteosarcopenia. Since known risk factors do not fully explain the occurrence of osteosarcopenia, the search for new causal factors, as birth weight (BW) is promising. **Objectives:** To evaluate whether BW was associated with DXA-determined body composition, bone densitometry and cardiometabolic risk markers in young women from the NutriHS. Paper 1 objective: to propose reference values for DXA-determined VAT, and to test their ability to identify the cardiometabolic risk profile. Paper 2: to examine whether BW was associated with muscle and bone DXA-determined parameters. Paper 3: to investigate whether parameters of muscle and bone compartments were associated and possible predictive factors of these compartments throughout life. **Methods:** NutriHS is a cohort study conducted in undergraduates and Nutrition graduates and here cross-sectional analyses were performed in 201 healthy women (20-45 years). They answered questionnaires and had anthropometry, muscle strength and performance, DXA-determined body composition and bone densitometry obtained. A random sample of 148 participants had also laboratory tests collected. Multiple regression models, using the directed acyclic graphs-recommended adjustments, were employed. **Results:** Median age was 23 years and mean BMI was 22.9±2.9 kg/m^2. Paper 1: Mean VAT mass and volume were 221.0±306.1 g and 231.8±323.8 cm^3, respectively. The third tertiles of VAT were significantly associated with increased frequencies of abnormal anthropometry, HOMA-IR and TyG indexes. Paper 2: Mean BW was 3,199±424 g; BW in quartiles was significantly associated with several muscle and bone parameters. Paper 3: Direct, strong and independent associations between bone and muscle variables were detected. **Discussion:** Cutoffs for DXA-derived VAT mass (221.0 g) and volume (231.8 cm^3) are being suggested for Brazilian young women; these seem able to disclose a mild visceral fat accumulation, prior the deterioration of glucose and lipid metabolism. The role of BW as an early marker for muscle and bone states in young adulthood was shown. In addition, a musculoskeletal profile for a healthy stratus of the Brazilian women was firstly described. Our findings indicated a muscle-bone crosstalk even in young adults and suggested predictive factors (such as BW, physical activity, smoking) of muscle and bone compartments.

Key words: DOHaD - birth weight - DXA - body composition - visceral adipose tissue - osteosarcopenia - bone mass - muscle mass - cardiometabolic risk

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ABBREVIATIONS

ABRASSO, Associação Brasileira de Avaliação Óssea e Osteometabolismo
AIDS, acquired immunodeficiency syndrome
A/G, android-to-gynoid ratio
ASM, appendicular skeletal muscle mass
ASMI, appendicular skeletal muscle mass index
AUC, area under the curve
AWGS, Asian Working Group for Sarcopenia
BMC, bone mineral content
BMD, bone mineral density
BMI, body mass index
BRADOO, Congresso Brasileiro de Densitometria, Osteoporose e Osteometabolismo
BRAZOS, The Brazilian Osteoporosis Study
BW, birth weight
CC, calf circumference
CVD, cardiovascular disease
CT, Computed tomography
CBR, Colégio Brasileiro de Radiologia
DAG, Directed Acyclic Graphs
DOHaD, Developmental Origins of Health and Diseases
DXA, dual-energy x-ray absorptiometry
e-NutriHS, electronic NutriHS (software)
ESPEN, European Society of Clinical Nutrition and Metabolism
EWGSOP, European Working Group on Sarcopenia in Older People
FMI, fat mass index
FNIH, Foundation for the National Institutes of Health
HOMA-IR, homeostatic model assessment- insulin resistance
ICFSR, International clinical practice guideline for sarcopenia
IOF, International Osteoporosis Foundation
IQR, interquartile range
ISCD, International Society for Clinical Densitometry

ISI, International Sarcopenia Initiative
IWGS, International Working Group on Sarcopenia
LS, lumbar spine
MRI, magnetic resonance imaging
NCCD, non-communicable chronic diseases
NF, neck femoral
NHANES, The National Health and Nutrition Examination Survey
NIH, National Institute of Health
NutriHS, Nutritionists' Health Study
ROC curve, receiver operating characteristic curve
ROI, region of interest
SD, standard deviation
TF, total femur
TFM, total fat mass
TyG, product of triglycerides and fasting plasma glucose
TyG-BMI, product of triglycerides and fasting glucose divided by BMI
TyG-WC, product of triglycerides and fasting glucose divided by waist circumference
VAT, visceral adipose tissue
VIGITEL, sistema de vigilância de fatores de risco e proteção para doenças crônicas por inquérito telefônico
WC, waist circumference
WHO, World Health Organization
WHtR, waist-to-height ratio

1. BACKGROUND

1.1. Developmental Origins of Health and Disease - DOHaD

Searching for new paradigms, epidemiological, clinical, experimental and translational studies have associated environmental insults occurred during pre-conception period, fetal life and at the beginning of extrauterine life with the development of NCCD (1). This science field has been named *Developmental Origins of Health and Disease* or DOHaD.

Initial reports of this theory date from 1930's decade, when investigators found that intrauterine conditions and infancy events were associated with survival of the offspring (2). Years later, the observation that offspring from mothers exposed to scarce food and poor nutrition during the first semester of pregnancy showed higher incidence rate of obesity than the offspring of mothers exposed to starvation only during the last trimester, reinforced the importance of intrauterine life (3).

Late 1980's, Barker and cols. proposed the theory of DOHaD based in the fetal programming to adapt to adverse conditions (4). They observed that the exposure to adverse circumstances during intrauterine life and infancy elevated the risk of cardiovascular diseases (5). Further studies reinforced that individuals with low birth weight showed higher risk of developing systemic hypertension, type 2 diabetes mellitus, disturbance of lipid profile and depression in adulthood (6-9). Such findings originated the thrifty phenotype hypothesis, in which the fetus would be able to adapt to a hostile maternal environment by optimizing energy supplies for his/her survival, prioritizing noble organs. Consequently, permanent alterations would occur in organs and systems that could influence risk of diseases in the life course (10). However, the thrifty phenotype theory did not clarify how and why such metabolic adaptations occurred since they would not be advantageous to that very moment (1). They were further explained by the *model of predictive adaptive responses*: the developing organism responds to an environmental challenge by producing a selectively appropriate phenotype, predicting physiological needs for survival in the future environment (11). Whether this later developmental environment match the predicted one, the diseases' risk is low, while mismatch is associated with increased risk of NCCD (11).

Therefore, DOHaD is based on the concept of plasticity of the development, i.e. the ability of a genotype to generate different morphological and physiological states induced by different early-life exposures (12). This adaptation is possible only during a critical period of development which, for most organs and systems, is limited to intrauterine life.

Epigenetics is the science that has explained environmental stimuli during the development process modify genes expression without altering DNA structure (13). These new characteristics, resultant from modified gene expression, are transmitted from generation to generation; the chapter of epidemiology in charge of this knowledge has been called intergenerational epidemiology (14). An observation that the offspring birth weight is closely associated with the mother's birth weight is indicative of fetal programing across generations (15).

Deepening the knowledge in intergenerational epidemiology is relevant considering the aging of populations and need to improve detection of high-risk individuals to NCCD. Investigations in the DOHaD field are promising to understand risk factors and mechanisms that could help in planning preventive measures against prevalent diseases in populations worldwide.

1.2. Epidemiology of body composition compartments through life

Brazilian aging has been attributed to reduction in fecundity rates, diagnostic and therapeutic advances among other factors. It is estimated that by 2050 the percentual of individuals older than 60 years will be 30% (16). Such greater longevity has modified the epidemiological profile, with increased morbidity and mortality due to NCCD (17,18).

Alterations in body composition are characteristics of the aging process, such as decrease in muscle and bone mass associated and increase in visceral and muscle fat deposition (19). The first two conditions are commonly associated with falls, fractures, hospitalizations, disabilities and elevated mortality (20-22). Central obesity is closely related to increased risk to metabolic and cardiovascular diseases (23) and when associated with low muscle mass can reduce muscle strength by inflammatory and endocrine pathways (24).

- *Muscle and bone compartments*

- *Sarcopenia*

Sarcopenia is a condition of clinical and functional importance and has been considered as a geriatric syndrome (25,26) and recognized as a disease by the World Health Organization in the International Statistical Classification of Diseases and Related Health Problems (27). The term sarcopenia, from the Greek sarx (meat) and penia (loss), was originally described by Rosenberg as the loss of lean mass due to aging (36). Current definitions of sarcopenia, however, are not limited to assessing the amount of lean mass, also considering muscle strength and performance (25, 37).

From the original study by Baumgartner et al for assessing muscle mass (38,39), several consensuses arose with the expectation of defining diagnostic criteria for sarcopenia, as summarized in Table 1 (25, 32, 37, 40-44). In 2010, the European Working Group on Sarcopenia in Older People (EWGSOP) defined sarcopenia as loss of muscle mass associated with reduced strength and/or muscular performance in elderly population (25). Years later, the Society for Sarcopenia, Cachexia and Wasting Disorders Group proposed that age would not be a condition for its occurrence and diagnosis (45), further accepted by the revised EWGSOP2 considering that "*the development of sarcopenia is now recognised to begin earlier in life*" (37, 46). This considers primary sarcopenia when no other specific cause is evident, and secondary when causal factors are detected such as AIDS, diabetes mellitus, unhealthy lifestyle, hormonal disorders etc (47, 48, 49-56). The EWGSOP2 recognized that strength is better than mass in predicting adverse outcomes (57, 58) and made recommendations for diagnosis (59).

Table 1. International consensus for definition and diagnosis criteria of sarcopenia.

International working group	Recommendations for diagnosing sarcopenia	Notes
European Working Group on Sarcopenia in Older People (EWGSOP) – 2010 (25)	"Both low muscle mass and low muscle function (strength or performance)", assessed in clinical practice using: - DXA, BIA, or anthropometrics; - grip strength; and - gait speed, SPPB, or TUG, respectively	The EWGSOP was the first consensus to define diagnostic criteria for sarcopenia, since it was the most used.
International Working Group on Sarcopenia (IWGS) – 2011 (42)	"Low whole-body or appendicular fat-free mass (measured using DXA) in combination with poor physical functioning (defined as gait speed <1m/s)".	Patients who are bedridden, cannot perform a chair rise, or with gait speed <1m/s should undergo DXA measurement, and sarcopenia diagnosed using validated definitions
Asian Working Group for Sarcopenia (AWGS) – 2014 (43)	"Low muscle mass plus low muscle strength and/or low physical performance"	Similar to the EWGSOP working definition, although using cut-off points specific to older adults from/descendent from South-East Asia
Foundation for the National Institute of Health (FNIH) – 2014 (40)	As per the EWGSOP definition, using DXA, gait speed and grip strength for measurement of LBM, muscle strength and physical performance respectively	Based on a detailed evaluation of clinically relevant cut-off points for weakness and low LBM.
International Sarcopenia Initiative (ISI) – 2014 (32)	As per IWGS and EWGSOP definitions	Formed by international experts from the EWGSOP and IWGS
European Society of Clinical Nutrition and Metabolism (ESPEN) – 2017 (44)	Endorsement of the EWGSOP diagnosis	Highlights that diagnostic criteria for sarcopenia have not yet been fully established
Sarcopenia: revised European consensus on definition and diagnosis. (EWGSOP2) – 2018 (37)	According to revised EWGSOP2: - sarcopenia is identified by low muscle strength; - sarcopenia diagnosis is supported by additional documentation of low muscle quantity and/or quality; and - severe sarcopenia is diagnosed by physical performance ability	The revised EWGSOP2 placed muscle strength in the centre of the diagnostic process, as opposed to muscle mass (23).
International clinical practice guideline for sarcopenia (ICFSR) – 2018 (41)	The ICFSR recommended the EWGSOP and FNIH definitions.	-

Adapted from J Nutr Health Aging 2018; 1-14.

DXA: dual-energy x-ray absorptiometry; BIA: bioimpedance; SPPB: Short physical performance battery; TUG: Timed-up and go test; LBM: lean body mass.

In the etiopathogenesis of sarcopenia there are modifications in the biology of skeletal muscle through metabolic, cellular, vascular and inflammatory mechanisms (60-67). It is a major component of the fragility syndrome and a strong predictor of disability, morbidity, hospitalization, and mortality in the elderly (68). However, changes in the muscle compartment should not be interpreted as a condition that begins in advanced age, but as a continuous process of life. Most studies have investigated the etiology of sarcopenia in advanced age, although earlier factors are determinant of peak muscle mass and strength, and consequently of future loss rate (46,69).

- *Osteoporosis*

National Institute of Health (NIH) consensus defines osteoporosis as a progressive systemic skeletal disease characterized by impairment of bone resistance, with an impact on bone density and quality, and a consequent increase in bone fragility and susceptibility to fracture (70). This is a major public health problem (71), that affects more than 200 million people worldwide (72). Approximately 30% of all postmenopausal women have osteoporosis in the United States and in Europe, and at least 40% will suffer one or more fragility fractures (more frequently in the vertebrae, distal radius, and proximal femur), causing physical incapacity and reducing the quality and life expectancy (73, 74). The profile of osteoporosis in Brazil has been reported (75-78). The BRAZOS evaluated 2,420 adults in five Brazilian geographic regions and verified that sedentary lifestyle, smoking, poor quality of life and diabetes in men, and advanced age, early menopause, sedentary lifestyle, poor quality of life, higher phosphorus ingestion, diabetes, falls, chronic use of benzodiazepines, and family history of hip fractures after 50 years of age in first-degree relatives in women were associated with low-impact fractures (75). The same investigators observed a high frequency of osteoporotic vertebral fracture in low-income community-dwelling elderly (76) and association with decreased quality of life (77).

Bone mineral density (BMD) decreases with aging; primary osteoporosis occurs in women mainly after menopause and in men around 75-80 years old (79). Secondary osteoporosis is less common and requires investigation in patients who suffer a fragility fracture without traditional risk factors, especially in young people

(80). The International Society for Clinical Densitometry does not recommend bone densitometry routinely in young women (81).

- *Osteosarcopenia*

In recent years, knowledge of crosstalk between bone and muscle and clinical observation of the concomitant occurrence of sarcopenia and osteoporosis call attention to the importance of considering muscles and bones as a functional unit (82,83) and motivated the term *osteosarcopenia*. There is evidence that both tissues start growing early in life, parallelly until reaching their peaks around 25-30 years of age (84). Interestingly, cycles of muscle strength and muscle and bone masses are very similar (**Figure 1**). The integrity of the biochemical and biomechanical systems in such musculoskeletal unit is essential to guarantee a healthy aging. Muscle and bone masses are maintained during adulthood, and their deterioration rates depend on several genetic, epigenetic and environmental factors (82, 85).

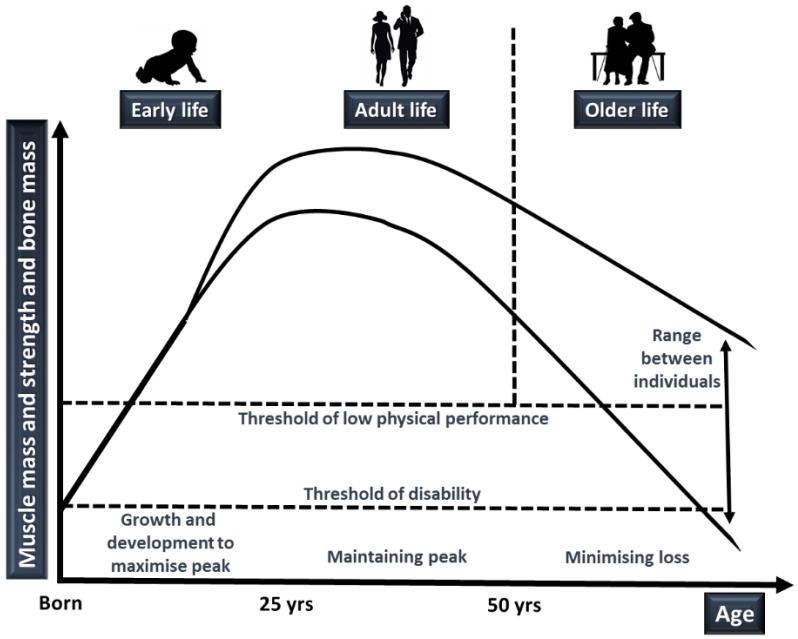

Figure 1: Muscle mass and strength, and bone mass and the life course. To prevent or delay osteosarcopenia development, maximize muscle and bone in youth and young adulthood, maintain in middle age and minimize loss in older age. Original from Sayer AA *et al* (46) and adapted from Cruz-Jentoft AJ *et al* (37).

Publications has increased especially in basic areas focusing in biochemical and biomechanical mechanisms involved in musculoskeletal interaction (82, 86-90).

Co-existence of osteoporosis and sarcopenia or osteosarcopenia (91, 92) is a chronic condition, typically manifested in frail elderly population and associated with disability and mortality (93). Despite sharing common risk factors and pathophysiological pathways (94), they do not fully explain their occurrence, justifying the search for new causal factors such as those present in early stages of life (82).

Aging cohorts evaluated the association between early life factors and muscle compartment in elderly individuals. An association between low birth weight and lower palmar grip strength when aging was reported (95, 96). The U.K. National Birth Cohort observed a strong relationship between birth size and palmar grip strength in adults born in 1946 (97). However, other studies found that muscle mass in the elderly was positively associated with birth weight (BW) regardless of birth size (98, 99). Long periods of breastfeeding were also associated with higher palmar grip strength in men from the Hertfordshire Cohort Study (95).

Studies have shown association between low BW and reduction of muscle mass and/or strength in elderly individuals when imminent risk of sarcopenia is present. In recent years, birth cohorts investigated the relationship between BW and changes in the muscle compartment in children and adolescents. A systematic review and meta-analysis of thirteen studies, of which nine involving individuals under 40 years, showed that each extra kilogram of BW was associated with a mean increase of 0.86 kg in palmar grip strength after adjustment for age and height (100). Similar positive association with lean mass was observed in this population (101). In addition, evidence that long-term breastfeeding was associated with increased muscle strength in adolescents reinforced the potential contribution of diet in achieving adequate peak and muscle strength (101). Based on these findings, low BW and growth deficits during childhood and adolescence has been considered risk factors for sarcopenia (102).

Regarding bone mass, a systematic review and meta-analysis demonstrated a positive association between BW and bone mass among children although weak in adults (103), concluding that BW may favor bone health in later life. On the other hand, a European birth cohort verified that prenatal growth had no significant impact

in total body and lumbar spine bone mineral density (BMD) in early adulthood (104), but the weight gain during childhood (105). Similarly, the Helsinki Birth Cohort Study evaluating elderly women also concluded that information on early growth did not improve prediction beyond that predicted by current height and weight (106). In a birth cohort conducted in Brazil, BW predicted bone mass assessed by densitometry in adults at age 18 years (107). Such association was confirmed in young women (108).

These considerations highlight the importance of evaluating muscle mass, strength and function, as well as bone parameters in young individuals.

- *Adipose compartment*

The impact of NCCD on public health is widely recognized. WHO estimates that NCCD accounted for 71% of the world's deaths in 2016 (109); in Brazil, were responsible for 72.7% of all deaths in 2011 (110). According to VIGITEL (2017) the number of overweight people grows: 54.0% of Brazilians are overweight and between 15.0 and 23.8% of the population is obese (111). These findings are in agreement with American data, showing a significant increase in obesity among women aged 60 years and over (112). This scenario coupled with the aging population culminates with the emergence of a new challenge for public health, the sarcopenic obesity. *Sarcopenic obesity* consists of the coexistence of sarcopenia and obesity, that is, in the combination of low lean mass and high body fat mass (113). This clinical entity, i.e. "the confluence of two epidemics", determines a synergism for the worsening of metabolic and physical functions, resulting in great functional disability (114).

The pathogenesis of sarcopenic obesity is not completely elucidated, although the existence of an interaction between abnormalities in muscle and adipose tissues. Changes in the muscle compartment (generated by sarcopenia) and fat infiltration (secondary to weight gain) are believed to trigger inflammation. Physical inactivity and other factors contribute to increased body adiposity, inflammatory adipokine production and insulin resistance, worsening the state of subclinical systemic inflammation. A vicious cycle of loss of lean mass and increase of adipose tissue perpetuates the occurrence of sarcopenia and sarcopenic obesity (115).

In this context, another risk association is the so-called *dynapenic abdominal obesity*, dynapenia and visceral obesity; the concomitance of these conditions provokes disabilities in routine activities (116).

Obesity raises the risk of hypertension, dyslipidemia, type 2 diabetes and cardiovascular diseases (CVD) and combinations of these risk factors are named metabolic syndrome (117,118).

In the last decades, scientific community has searched for factors that identify at-risk individuals for cardiometabolic outcomes at an earlier stage. The role of visceral adipose tissue in producing inflammatory cytokines represent an important pathophysiological mechanism (119). More recently, the knowledge of intestinal microbiota has contributed to understand the cardiometabolic risk pathway (120). Despite this knowledge, these factors do not explain the totality of outcomes. In this scenario, studies have emerged in an attempt to detect at-risk individuals earlier, during their course of life.

In the Hertfordshire Cohort, one-year-old weight was strong and inversely associated with obesity and CVD in adult life (121). Similarly, the Helsinki Cohort observed that men who developed obesity and CVD have had low BW, remained with low BW in childhood and had gained considerable weight after 5 years of age (122).

Prevalence of overweight and obesity in the Brazilian pediatric population, has reached, respectively, 33.5% and 14.3% among children aged 5-9 years, and 20.5% and 4.9% between 10-19 years (123). Excessive nutrition in the pre- and postnatal periods has been associated with obesity in childhood and adulthood and with the occurrence of metabolic syndrome later in life (124-127). A Brazilian cohort of 468 children at birth and between 5-8 years of age observed that those with a higher BW had increased risk for obesity and other chronic diseases in adult life (128). In the same cohort, healthy 5 to 8-year-old children who had had lower BWs and higher waist circumferences showed increased insulin resistance (129). Several studies have reinforced that excessive weight gain in the first year of life is a predictor of subsequent increase in abdominal fat deposition (130, 131). Recently, it was reported that children with greater BW, with rapid weight gain in childhood and those whose mothers were obese, tended to be at greater risk of obesity (132). In Brazil, associations of BW and weight gain rates during different developmental periods (0-6 months, 6-12 months, 12-24 months, 2-5 years and 5-10 years) with overweight and obesity at 10 years of age were investigated in 147 children from a low-income region of São Paulo (133). The rate of weight gain during the first 6 months of life and

between 2-5 years of age, and the weight at 5 years of age were important predictors of overweight or obesity at 10 years of age.

1.3. Body composition assessment: Dual energy X-ray absorptiometry

Compilation data from cadaveric dissection culminated in the so-called *reference men*, which represents the entire human body and has served as basis for the development of methods for assessing body composition (134,135).

The Dual X-ray Absorptiometry (DXA) dual-emission method is based on the attenuation difference of the ionizing energy between the bone tissue and the soft tissues (136). It is characterized by being an atomic-level, tricompartmental method, analyzing in an individualized way the bone, lean and fat masses. This is non-invasive method to evaluate the total body for all age groups but contraindicated for pregnant women. In validation process, DXA was compared with the *reference man* and a strong correlation was observed for fat mass (r^2 0.88, $p < 0.01$), as well as for muscle mass (r^2 0.74, $p < 0.01$), indicating its utility for clinical purposes (137).

Computed tomography (CT) and magnetic resonance imaging (MRI) are direct methods of evaluating body composition, considered gold standards (138). Compared to MRI, DXA limitation is the inability of measuring ectopic fat deposits in organs and intramuscular and epicardium adipose infiltrations (139-142). However, DXA advantages are low radiation level and cost, and faster acquisition of images (138). The DXA technique, combined with adequate softwares, has allowed individualized analysis of muscle, adipose and bone compartments. Therefore, DXA has been largely employed in clinical setting and research.

- *Muscle compartment*

Appendicular skeletal muscle mass (ASM), calculated by the sum of the lean mass of arms and legs are parameters provided by the DXA (143). Using the ASM, Baumgartner et al. proposed an estimate of relative skeletal muscle mass, the so-called Appendicular Muscle Mass Index (ASMI) or Baumgartner Index (38). ASMI is obtained by dividing ASM (in kg) by the height squared (in meters) and has been the most widely used index to assess sarcopenia. Borderline values for elderly individuals were defined as two standard deviations from the mean of the reference data for young adults (18-40 years), ≤ 7.26 kg/m^2 for men and ≤ 5.45 kg/m^2 for women. In

Brazil, the study Health, Welfare and Aging – SABE (in Portuguese, *Saúde, Bem-Estar e Envelhecimento*) used cut-off values for ASMI in the elderly population of ≤ 8.90 kg/m^2 for men and ≤ 6.37 kg/m^2 for women (33), using the Lee equation (144).

After evaluating two different approaches to adjust lean mass for body size, ASM adjusted for height and for body fat mass (145), Newman et al. suggested that "fat mass should be considered in estimating prevalence of sarcopenia in women and in overweight or obese individuals". In 2014, the Foundation for the National Institutes of Health Biomarkers Consortium Sarcopenia Project suggested a new index for adjusted ASM by dividing it by the BMI (146). Thus, cutoffs proposed for ASMI were < 0.789 for men and < 0.512 for women, which should be used only for individuals aged 65 years and over.

Baumgartner *et al*. had the opportunity of evaluating 229 non-Hispanic White individuals aged 18-40 years, using ASMI cutoffs of ≤ 8.60 kg/m^2 for men and ≤ 7.30 kg/m^2 for women (38). A study including young Mexicans for establishing cutoffs for the ASMI observed values lower than those of non-Hispanic American youth (147). The authors emphasized this aspect since the diagnosis of sarcopenia would be underestimated in Latin American elders if Baumgartner's criteria were used. A Brazilian study conducted in 500 women aged 20 to 84 years verified that the ASMI was smaller than those observed in Americans involved in NHANES III in almost all ages and ethnic groups (148). These findings emphasize the importance of establishing specific cutoffs for different populations.

- *Bone compartment*

BMD measured by dual X-ray absorptiometry is the gold standard to diagnose osteoporosis (149). WHO defines osteoporosis when T-score ≤ -2.5 and osteopenia when between -1.1 and -2.4 and recommended femoral neck and lumbar spine as the anatomic regions of interest (ROI) (150). The Z-score is recommended for comparison with control population of the same sex and age (151). In young women, low bone mineral density or content (BMD or BMC) are define as a Z-score ≤ -2.0 adjusted for age, gender and body weight (152) Secondary osteoporosis has been attributed to the condition of bone density Z-score of –2.5 or less (153).

- *Adipose compartment*

Despite widely used to estimate body adiposity (154), BMI measures weight excess but not excessive fat directly nor its distribution (155). Waist circumference (WC) as well as its ratio with hip or height (WHtR) have been shown more effective to demonstrate association of adiposity and cardiometabolic risk (156, 157).

High accurate assessment of body adiposity requires specific techniques such as the DXA. This provides measurements of the total fat mass (TFM), from which the fat mass index (FMI) is calculated, by the TFM quotient (in kg) by the square of the height (in meters) (158). A classification of body adiposity was proposed in which FMI values between 3.0 and 6.0 for men and between 5.0 and 9.0 for women have been considered normal (**Table 2**) (158).

Table 2: Classification of body adiposity according to the fat mass index in each sex. Adapted from Kelly TL et al (158).

FAT MASS INDEX FMI (kg/m^2)	Men	Women
Severe deficiency	< 1.99	<3.49
Moderate deficiency	2.00 – 2.29	3.50 – 3.99
Mild deficiency	2.30 – 2.99	4.00 – 4.99
Normal	3.00 – 6.00	5.00 – 9.00
Overweight	6.10 – 9.00	9.10 – 13.00
Obese class I	9.10 – 12.00	13.10 – 17.00
Obese class II	12.10 – 15.00	17.10 – 21.00
Obese class III	> 15.10	>21.10

DXA also allows the analysis of body fat distribution, discriminating fat from android and gynoid regions (**Figure 2**) and allowing the calculation of the android-to-gynoid fat ratio – A/G (159). Recommended A/G values are < 1; predominance of android fat is defined by the A/G > 1, which is interpreted as unfavorable cardiometabolic profile (159).

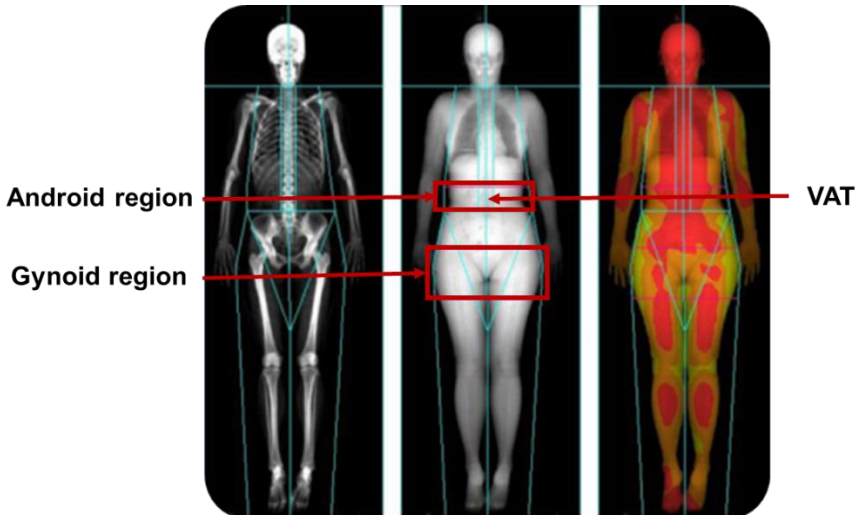

Figure 2: Anatomical representation corresponding to DXA-determinated android and gynoid regions and visceral adipose tissue (VAT) area.

Another valuable information provided by DXA is the estimation of the visceral adipose tissue – VAT (**Figure 2**). A comparison of findings of DXA-VAT with computed tomography (measured at L4-L5 level) showed a significant correlation between methods for assessing visceral fat in a population aged 5 to 18 years. Both methods had a similar association with cardiometabolic risk factors (160). DXA-VAT was obtained from 120 healthy Caucasian women aged 20 to 40 years, aiming to determine normal range of VAT and its correlation with cardiometabolic risk factors. VAT corresponded to 0.37 ± 0.3% of body weight and 1.11 ± 0.72% of body fat. The averages of VAT in grams and volume were 235.9 ± 183 g (95% CI 202.7-269.1) and 250.3 ± 194.5 cm^3 (95% CI 215.1-285.4), respectively. Authors also found a moderate correlation of VAT with anthropometric measures, HDL-cholesterol (r = **-** 0.193, p = 0.03), glycemia (r = 0.252, p = 0.005) and HOMA (r = 0.184, p = 0.049) (161). In a study conducted in Caucasian and African-American obese women aged 21-69 years, DXA-VAT was predictive of glucose intolerance and metabolic syndrome (162).

Based on these data, DXA-VAT has been considered an appropriate method to assess visceral fat and, consequently, to predict cardiometabolic risk.

1.4. Nutritionists' Health Study

Although nutritional, demographic and epidemiological transitions have led to high prevalence of NCCD in developing countries, studies with a focus in the DOHaD theory is lacking. Search of new risk factors or markers present in early phases of development, that could help understanding and preventing these diseases are of great interest. In this context, the **Nutritionists' Health Study - NutriHS**, represents an opportunity to fill some gaps in this knowledge.

NutriHS is a cohort study conducted in undergraduates and graduates of Nutrition courses from the School of Public Health of the University of São Paulo (FSP/USP) and other institutions. It was approved by the institutional ethics committee (COEP # 991,542 / 15 and 257,513 / 13) (Attachment 1) and follows norms of the National Health Council, regarding ethics in research with humans (163).

A specific software (*e*-NutriHS) was developed to obtain the database that enables continuous insertion of data, immediate storage and updating of the participants' information and allows dialogue with in statistical packages.

Main NutriHS objectives have been:

- To analyze life habits (diet, physical activity, smoking), anthropometric profile and body composition throughout the course of Nutrition;
- To evaluate the profile of traditional cardiometabolic risk factors and new markers and their association with body composition;
- To analyze the composition of the gut microbiota and its association with eating habits and with a cardiometabolic risk profile;
- To analyze the association of informed pre-pregnancy maternal characteristics, gestational and early childhood data with a cardiometabolic risk profile, with emphasis on body composition and gut microbiota.

2. RATIONALE OF THE CURRENT STUDY

The lifestyle of young populations has been characterized by physical inactivity, unhealthy dietary habits, use of indoors technological facilities and low sun exposure. This scenario contributes to a negative impact in their body composition, favoring decreases in muscle and bone masses and increase in adipose tissue. This condition has been associated with elevated risk for several NCCD with aging.

Early assessment of their body composition using accurate methods is important to screen young adults of higher risk of abnormalities in muscle, bone and adipose sites in order to plan more effective interventions.

Also, it is relevant understanding additional factors associated with risk of lower muscle and bone parameters, as well as greater body adiposity, such as previous maternal conditions and early-life events. It was unclear, whether slight alterations in these parameters could already be influencing biochemical outcomes in youngsters.

The NutriHS offers a unique opportunity to obtain high-quality data regarding early phase of development of undergraduates and graduates in Nutrition. It is expected that these participants provide health-related data to be associated to outcomes in adulthood. The availability of DXA and biochemical parameters allows accurate assessment of body composition and bone densitometry and testing associations with selected outcomes in young adults. Late diagnoses of sarcopenia and/or loss of bone mass, as well as visceral adiposity, limit the efficacy of therapeutics. Therefore, it is of great interest early identification of at-risk individuals considering the potential to more effective preventing programs against NCCD. The prospective design of the NutriHS will permit explore hypothesis to be raised from the present study.

3. OBJECTIVES

3.1. GENERAL OBJECTIVE

To evaluate whether BW was associated with DXA-determined body composition, bone densitometry and cardiometabolic risk markers in young women from the NutriHS.

3.2. SPECIFIC OBJECTIVES

Considering that this PhD thesis is composed by three papers, specific objectives corresponded to those of each paper.

Paper 1 is entitled “Proposal of dual-energy x-ray absorptiometry values of visceral adipose tissue for Brazilian young women: NutriHS”. This aimed to propose reference values for DXA-determined VAT mass and volume, and to test their ability to identify the cardiometabolic risk profile in healthy young women, participants of the NutriHS

Paper 2, entitled “Birth weight is associated with dual-energy x-ray absorptiometry-determined muscle-bone unit in young healthy women from the Nutritionists’ Health Study”, aimed to examine whether BW was associated with the muscle-bone unit using DXA-determined parameters in young healthy women from the NutriHS.

Paper 3, entitled “Muscle-bone relationships and associated factors throughout life in Brazilian healthy young women from the Nutritionists’ Health Study”, investigates in young healthy women from NutriHS: 1) whether parameters of muscle and bone compartments were associated; and 2) examine predictive factors of muscle and bone parameters related to events from the early life and current habits.

4. METHODS

4.1. DESIGN & SUBJECTS

The current cross-sectional analysis was performed with the baseline data of the NutriHS. Recruitment and data collection occurred from September 2015 to April 2018. A convenience sample of the first 201 participants who met eligibility criteria was taken.

The inclusion criteria were: age between 20 to 45 years, female sex, regular menstruation, no past or current history of weight change > 5% of total body weight in the last year, malignancies, abnormal blood pressure levels and disturbances of glucose and lipid metabolism. Participants who were born premature, from gemelar pregnancy, who were pregnant or whose anthropometric measures exceed the limits of the densitometer (1.87 m and/or 201 kg, respectively) were excluded.

Participants of this sub-study of NutriHS have signed, electronically, the original informed consent form which includes the current procedures (Attachment 2).

4.2. PROTOCOL

The NutriHS research protocol had three steps, which were detailed elsewhere (164).

- Step I:

Using personal contacts in Nutrition colleges and social networks, subjects interested in knowing about the NutriHS were invited to access the page linked to the website of FSP/USP (www.fsp.usp.br/nutrihs) and to sign Informed Consent Form if agreed in participating. Then, they were able to log-in and fill online questionnaires related to (Attachment 3):

- Sociodemographic data;
- Clinical data;
- Dietary assessment (165);
- Physical activity assessment (166,167);
- Maternal data and early life factors (pregnancy, birth-related and childhood data).

After completing the questionnaires, participants were invited to schedule page a first clinical visit, in which anthropometric assessment, stool sample delivery and blood collection would be performed.

NutriHS researchers make the online conference of data electronically collected to identify and correct inconsistent responses. Directions for the clinical visit and the collection were e-mailed.

- *Step II:*

At the health center of the School of Public Health, University of Sao Paulo, participants underwent blood pressure and anthropometric measurements and biological sample collections. The body weight was obtained on a digital scale with precision of 100 g and the height in a wall stadiometer with a precision of 0.1 cm. Body mass index was calculated by dividing weight by squared height. Waist circumference was obtained using a flexible and inelastic tape, with a precision of 0.1 cm, locating the midpoint between the last rib and the iliac crest. Blood pressure levels were taken in triplicate using an automatic device (Omron model HEM-712C®, Omron Health Care Inc, USA), with an appropriate cuff to the brachial circumference, in sitting position, after 5-minute rest. Mean values of the last two measurements were considered for final systolic and diastolic blood pressure levels. Blood sample was collected after overnight fasting. Part of the biochemical analyses was locally performed, and other was stored at -80ºC for later analyses.

Lipid profile (total cholesterol, HDL-c and triglycerides) was analyzed by colorimetric and enzymatic methods and LDL-c calculated by the Friedwald equation. Plasma glucose was obtained by the glucose oxidase and insulin by Immunofluormetric assay, based on monoclonal antibody (Monobind, Inc, Lake Forest, CA, USA). Insulin resistance was estimated by the HOMA-IR index (168). 25-hydroxyvitamin D levels were measured by electrochemiluminescence assay on Elecsys analyzers and Cobas modular platforms (Roche® Diagnostics, Indianapolis, USA) (169).

- *Step III:*

Participants were invited for a second visit for evaluation of body composition and bone densitometry. Procedures were made always by the same researcher (PhD candidate), certified by the Brazilian Association of Bone and Osteometabolism

(ABRASSO). Previously, they received information about preparation and restrictions (Attachment 4).

Data related to body composition were registered in a standardized form (Attachment 5). This visit also represented an opportunity to double-check information from questionnaires, mainly regarding early-life events and current lifestyle such as physical activity, smoking, alcohol consumption, use of medications and family history. Anthropometric measurements were repeated.

Muscle strength was evaluated by the palmar grip strength, using a manual dynamometer (Jamar®), according to technique previously described (170, 171). Measurements were made seated in duplicate in the dominant limb, respecting a one-minute interval to avoid muscular fatigue, and the mean value was considered. The participant was instructed to tighten the dynamometer with the highest possible force for three seconds and then release. The result was then read out.

Chair-stand test was obtained as a measure of legs' strength. Participant was asked to sit with arms crossed over the chest, and the time to get up and sit as fast as possible five times was measured (172). In gait speed test, a muscle performance test, the participant was oriented to walk normally on a 4-meter course; time was checked, and results provided in meters per second.

Body composition and bone densitometry were assessed by dual energy X-ray absorptiometry (GE Lunar iDXA®, Madison, WI, USA), following the manufacturer's recommendations. Initially, possible interferring factors were checked. For the acquisition of body composition, the subject was appropriately positioned within the limits of the equipment, with arms and legs extended along the body. The participant was repositioned to perform bone densitometry of the lumbar spine and femur. Results were e-mailed to participants.

According to the International Society for Clinical Densitometry (ISCD) recommendation (173) our precision values and least significant change (LSC) were calculated after three successive measurements in fifteen women, by the same researcher (PhD candidate). The LSC of our bone densitometry scans were: - lumbar spine BMD: 0.027 g/cm^2; - total femur BMD: 0.017 g/cm^2; and - femoral neck BMD: 0.056 g/cm^2. Precision values of body composition were: - total body BMD: 0.009

g/cm^2; - total body fat: 206.0 g and 0.3%; - total lean mass: 275.0 g; -appendicular skeletal mass: 350.0 g; - VAT volume: 25.8 cm^3 and - VAT mass: 28.5 g.

Statistical analysis

Data were presented as mean ± standard deviation (SD) or median and interquartile range (IQR). The normality of the variables was verified by the Kolmogorov-Smirnov test. For those with normal distribution, parametric tests (Student t test, Pearson's correlation and ANOVA) were used and for the other variables, non-parametric tests (Mann-Whitney, Spearman's correlation and Kruskal-Wallis) were performed. Multiple regression models were employed (linear, logistic or Poisson) to evaluate associations between exposures and outcomes. Minimal sufficient adjustments were defined according to the Directed Acyclic Graphs recommendations (174, 175) (www.dagitty.net).

In particular, Receiver Operating Characteristic (ROC) curve was employed to define VAT values able to identify the cardiometabolic abnormalities as dicotomic variables. Areas under the curve (AUC), sensitivity and specificity were provided for the classic risk factors. Poisson multiple regression was used to test independent associations of VAT (outcome variable, non-parametric) with exposures.

Statistical analyses were performed using the STATA 13.1® statistical package, and the level of significance was set at 0.05.

5. RESULTS

This chapter of the thesis has been replaced by three papers that are being submitted to publication. Therefore, their contents are arranged according to the requirements of each journal.

5.1. PAPER 1

Proposal of dual-energy x-ray absorptiometry values of visceral adipose tissue for Brazilian young women: the Nutritionists' Health Study

Running head: **DXA-VAT in Brazilian young women: NutriHS**

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ABSTRACT

BACKGROUND: Visceral adiposity is a risk factor for cardiometabolic diseases that can be associated with deterioration of the risk profile even in young adults. Dual-energy x-ray absorptiometry (DXA) is a precise method for measuring visceral adipose tissue (VAT); reference values need to be age-, gender- and race-specific. We proposed reference values for DXA-VAT and tested their ability to identify the cardiometabolic risk in Brazilian young women.

METHODS: In this cross-sectional analysis, 201 healthy young women reported sociodemographic data, early-life events and current characteristics, and underwent clinical examination and body composition using DXA. Blood pressure and laboratory tests were obtained for a random sample of 148 participants. ROC curve was used to define the VAT values able to identify the cardiometabolic abnormalities. Associations of DXA-VAT with clinical and laboratory data were tested by Poisson regression, adjusted according to directed acyclic graphs (DAG).

RESULTS: Sample had a median age of 23.0 years and normal BMI (22.9±2.9 kg/m^2). Mean VAT mass and volume were 221.0±306.1 g and 231.8±323.8 cm^3, respectively. VAT was correlated to BMI (r=0.60), waist (r= 0.57), WHtR (r=0.60) and TyG-derived variables (TyG-BMI: r=0.59 and TyG-waist: r=0.61). Greater VAT areas under the curves were observed for BMI, waist and WHtR when compared to those obtained for isolated biochemical risk factors. Regression models showed that third tertiles of VAT were significantly associated with increased frequencies of abnormal anthropometric indexes, triglycerides and HOMA-IR, adjusted for maternal pre-pregnancy BMI, birth weight, breastfeeding, age, skin color and physical activity.

CONCLUSION: DXA-values of 221.0g and 231.8cm^3 for VAT mass and volume, respectively, should be useful to identify the cardiometabolic risk in Brazilian young women. These cutoffs seem to be able to disclose mild visceral fat accumulation, prior glucose and lipid metabolism deterioration, reinforcing its role as an early cardiometabolic risk marker. Anthropometry utility for the identification of increased VAT was confirmed. Follow-up is needed to confirm the DXA-VAT ability to detect young at-risk individuals.

Key words: Visceral adipose tissue, DXA, DXA-VAT, cardiometabolic risk factors

BACKGROUND

Prevalence rates of obesity has increased worldwide. In 2016, 39% of adults had weight excess, and obesity was present in 11% of men and 15% of women (1). In particular, centrally distributed body fat represents a strong risk factor for cardiometabolic and malignancies in mid-to-late life (2,3). A recent study has indicated that increased body adiposity can deteriorate cardiovascular health even in young adults (4), reinforcing the importance of using accurate methods to measure visceral fat deposition for early identification of risk.

Despite the utility of body mass index (BMI) for the assessment of the nutritional status (5), it has flaws for not considering individual characteristics such as age, sex, race and distribution of body fat. Waist circumference (WC) as well as its ratio with hip or height (WHtR) have been shown more effective to demonstrate association of adiposity and cardiometabolic risk (6,7). Hypertrophy of visceral adipocytes induces insulin resistance – a pathophysiological basis for metabolic disturbances (8). Several indexes have been proposed to assess insulin sensitivity such as the HOMA-IR and the product of triglycerides and fasting glucose (TyG index) (9). Additionally, there is evidence that the combination of TyG index and an anthropometric measure (TyG-BMI or TyG-WC) may improve the prediction of type 2 diabetes and cardiovascular disease (9,10). These data are limited to some populations and such indexes have not been associated with direct measurements of visceral fat.

Computed tomography (CT) and magnetic resonance imaging (MRI) are considered gold standards for measuring visceral adipose tissue (VAT), but their disadvantages are radiation exposure and cost (11). Alternatively, dual-energy x-ray absorptiometry (DXA) represents a precise and safe method since radiation level is very low (12). Several studies have reported strong correlations between CT and MRI- and DXA-determined VAT measurements (13,14). However, few studies have suggested reference ranges and cut-off points for VAT using DXA to assess cardiometabolic risk. For our best knowledge, VAT reference values for young Brazilian population have not been proposed. This would be useful to anticipate prevention of metabolic and cardiovascular diseases. The Nutritionists' Health Study (NutriHS) represents a unique opportunity to explore possible cutoff values for VAT

by testing their association with markers of cardiometabolic risk in a young population (15).

In a sample of young women, participants of the NutriHS, this study aimed to propose reference values for DXA-determined VAT mass and volume, and to test their ability to identify the cardiometabolic risk profile in healthy young women.

METHODS

- **Study design, setting and participants**

The current cross-sectional analysis was performed with the baseline data of the NutriHS. The main study launched in 2014 includes undergraduates and graduates from Nutrition courses in São Paulo State, Brazil, and was approved by institutional ethics committee. All the participants signed the informed consent electronically, using the NutriHS website (www.fsp.usp.br/nutrihs). Details on the purposes and methodological concerns were reported elsewhere (16).

Recruitment and data collection occurred from September 2015 to April 2018. After having signed informed consent, participants answered online questionnaires. A researcher checked information provided and sent an e-mail with orientations to visit the health care center of the School of Public Health of the University of São Paulo. They were submitted to clinical examination and collection of biological samples. In a second visit, participants had body composition assessed by DXA.

For the present analysis, a convenience sample of the first 201 participants of NutriHS who met eligibility criteria was taken. Inclusion criteria were: age between 20 to 45 years, female sex, regular menstruation, no past or current history of important weight changes (> 5% of total body weight in the last year), malignancy, use of medications – except for contraceptives, abnormal blood pressure levels and disturbances of glucose and lipid metabolism. Exclusion criteria were: pregnancy and anthropometric measures exceeding the limits of the densitometer (1.87 m and/or 201 kg, respectively).

All of 201 participants included in the current study had body composition determined by DXA. A random subsample consisted of the first 148 participants included had blood pressure measured and biochemical data collected.

- **Variables**

Self-reported sociodemographic data, early life events and current characteristics of the participants were obtained from the questionnaires and used to build the theoretical model. Visceral adipose tissue mass and volume were outcome variables. Anthropometric variables were taken as exposures, while indexes of insulin resistance, blood pressure and biochemical variables (fasting plasma glucose, lipoproteins and triglycerides) were used to assure the association of VAT values with cardiometabolic risk factors.

- *Anthropometric and clinical data*

Weight was measured using with digital scale (Filizola®, São Paulo, Brazil) to the nearest 0.1kg and height with a fixed stadiometer with 0.1 cm precision. Body mass index (BMI) was calculated. Waist circumference was obtained at the midpoint point between the lowest rib and the iliac crest. Waist-to-height ratio (WHtR) was calculated dividing the waist circumference by the height.

Blood pressure was taken three times using an automatic device (Omron model HEM-712C, Omron Health Care Inc, USA), with an appropriate cuff, in sitting position, after 5-minute rest. Values considered for systolic and diastolic blood pressure were those the means of the last two measurements.

- *Biochemical data*

The lipid profile was determined by colorimetric enzymatic methods and LDL-c calculated by the Friedwald equation. Plasma glucose was obtained by glucose oxidase method and insulin by immunofluorimetric assay, based on monoclonal antibody (Monobind, Inc., Lake Forest, CA, USA). Insulin resistance was estimated using the HOMA-IR index (16) and the triglyceride and glucose index (TyG) (17) and its derivates, the TyG-BMI and TyG-WC indexes (18).

- *DXA-derived measurements*

All DXA (GE Lunar iDXA®, Madison, WI, USA) procedures were performed by the same examiner who is certified by the Brazilian Association of Bone Assessment and Osteometabolism (ABRASSO). Regions of interest (ROIs) were determined manually according to the manufacturer's specifications and the VAT was estimated automatically by CoreScan software (EnCore version 15.0). During the study period,

densitometer calibrations were daily performed. Other parameters extracted from body composition examination were total body fat (TBF), fat mass index (FMI), android fat, gynoid fat and android-to-gynoid fat ratio (A/G). Precision values calculated according to the International Society for Clinical Densitometry (https://www.iscd.org/) after three successive measurements in fifteen women were: - TBF: 206.0 g and 0.3%; - VAT volume: 25.8 cm^3 and - VAT mass: 28.5 g.

- **Statistical analysis**

Data are presented as means and standard deviations (± SD) or medians and interquartile ranges (IQR). Variables were checked for normality using the Kolmogorov-Smirnov test. Differences between the characteristics of the entire sample (n = 201) and the random sub-sample (n = 148) were assessed by Student t test or Mann-Whitney U test. Outcome variables (DXA-determined VAT mass and volume) showed non-normal distribution; their percentiles (P10, P25, P50, P75, P90) were described. Correlations of VAT with quantitative variables were tested by Spearman coefficient. Receiver operating characteristic curve (ROC) was also employed to define VAT values able to identify the cardiometabolic abnormalities as dicotomic variables. Areas under the curve (AUC), sensitivity and specificity were provided for the following risk factors: BMI $\geq$ 25 kg/m^2, waist circumference $\geq$ 80 cm, WHtR ≥ 0.5, systolic blood pressure ≥ 130 mmHg, diastolic blood pressure ≥ 85 mmHg, triglycerides ≥ 150 mg/dL, HDL-c < 50 mg/dL, fasting plasma glucose ≥ 100 mg/dL and HOMA-IR ≥ 2.0. Values of TyG and its derivates equal or above the third tertile were considered abnormal (TyG ≥ 8.4, TyG-BMI ≥ 198 and TyG-WC ≥ 658). In an attempt to assure the diagnosis of abnormal metabolic condition, risk factors (an anthropometric plus a biochemical one) were also combined for obtaining the ROC curves. To check the ability of DXA-determined VAT in identifying cardiometabolic risk, values were categorized in tertiles; the sum of the first and second tertiles considered reference. Poisson multiple regression was used to test independent associations of VAT with exposures, in which the directed acyclic graphs-recommended (DAG) minimal sufficient adjustments (19) (www.dagitty.net) were employed (Figure 1). Version 13.1 of Stata® statistical package was used, and level of significance was set at 0.05.

RESULTS

The sample was composed of young adults with a predominance of White women (72.4%) and a normal mean BMI. Comparison of the characteristics of the entire sample (n = 201) and random subsample (n = 148) showed that the groups were comparable (Table 1). Mean values of anthropometric indexes, blood pressure and biochemical data of the subsample were within the normal ranges.

Table 2 depicted mean (± SD), median (IQR) and percentiles of VAT for the entire sample and the subsample. Values of VAT mass and volume did not differ between groups.

VAT had strong correlations with anthropometric data (BMI: r = 0.60, waist circumferece: r = 0.57, and WHtR: r = 0.60) and TyG-derived variables (TyG-BMI: r = 0.59 and TyG-WC: r = 0.61). As expected, VAT was also correlated to other DXA-determined parameters (TBF: r = 0.72, FMI: r = 0.69, and A/G: r = 0.79). Correlation coefficients of VAT and biochemical variables were weaker, ranging from 0.18 to 0.30 ($p < 0.05$).

DXA-determined VAT cutoff points associated with abnormal anthropometric and laboratory parameters (predictors of cardiometabolic risk), AUC, sensitivity and specificity were shown in table 3. Greater VAT AUCs were observed for BMI, waist circumference and WHtR when compared to those obtained for biochemical risk factors in isolation. Combinations of an abnormal anthropometric variable with an abnormal biochemical risk factor resulted in increases in AUC and sensitivity and specificity (Table 3 and Figure 2).

Crude regression models for VAT mass and volume indicated significant associations with all markers of cardiometabolic risk except for HDL-cholesterol. In multiple regression models, the third tertiles of VAT mass and volume were significantly associated with increased prevalence of abnormal BMI, waist circumference, WHtR, triglycerides, HOMA-IR, TyG, TyG-BMI and TyG-WC, adjusted for maternal prepregnancy BMI, birth weight, breastfeeding, age, skin colour and physical activity, but not with diastolic blood pressure (Table 4). By adjusting for age or BW in isolation, the statistical significant association of VAT and diastolic blood pressure disappeared.

DISCUSSION

As far as we know, this is the first study to suggest cutoff values for DXA-derived VAT mass and volume in a sample of Brazilian young healthy women. Using ROC curves, our findings reinforced that anthropometry is a valuable tool for the identification of increased VAT, since anthropometric measurements (BMI, waist circumference and WHtR) or indexes (TyG-BMI and TyG-WC) corresponded to the best VAT AUCs. VAT thresholds suggested to identify of abnormal risk factors – particularly anthropometric parameters and abnormal TyG derivates – were between the 50th (103 g for mass; 108 cm^3 for volume) and 75th percentiles (274 g for mass; 289 cm^3 for volume) of DXA-derived VAT. Therefore, based on mean values found in the present study, young women with VAT mass of 221 g and volume of 232 cm^3 or above should be aware for cardiometabolic risk, even with normal biochemical profile. The knowledge of such values of visceral adiposity is desirable for early assessment of cardiometabolic risk but should not be extrapolated to other populations (20-22).

Differences in body composition related to sex, age, race and method of assessment limited the comparison of our study with others reported in literature. Our measurement of VAT using DXA and a specific software has been considered an accurate method and valid to assess visceral adiposity-related risk. A study including adults of both sexes from a Middle Eastern population to determine the DXA-derived VAT accuracy, showed high correlation with MRI ($r = 0.93$; $p < 0.001$ in women). Despite overestimating VAT, DXA average bias was low (+46.8 cm^3) and occurred especially when volume was > 750 cm^3 for women (23). Recent investigation examined the precision error of DXA-VAT with the same software used in our study and observed that mass measurements increased with BMI: for normal weight was 32.9 g, for overweight 33.5 g and for obesity: 51.0 g (24). Therefore, in obese individuals, the interpretation of DXA-VAT mass should take the total adiposity into consideration. Despite the inclusion of some overweight or obese women, the majority was eutrophic as expressed by the mean BMI of 22.9 kg/m^2.

In a study of 120 healthy Caucasian European women aged 20-40 years, similar methodological strategy was employed to evaluate the association of DXA-VAT and anthropometric measurements (25). A moderate correlation was detected, and mean DXA-determined VAT mass and volume were 236 g and 250 cm^3, respectively. These findings resemble ours, although we had observed stronger

correlation coefficients between VAT and anthropometric measurements. The same investigators evaluated 421 healthy adults of both sexes, aged 20 to 30 years (26). As expected, men had higher VAT than women. Mean VAT mass (258 g) and volume (273 cm^3) were higher than those found in women from our study. In contrast to our results, VAT values were strongly correlated to clinical variables (blood pressure, lipids, glucose and HOMA-IR), and greater AUC for triglycerides ≥ 150 mg/dL was found, suggesting that health conditions of participants may be worse than ours. Since our sample had lower deposition of visceral fat, milder abnormalities in risk factors were found, as well as weaker associations with biochemical parameters.

In our results, VAT AUC obtained when increased BMI was the risk factor reached the highest value despite being a measure of a total body adiposity. Also, sensitivity and specificity reached the best values. BMI is still considered a valuable predictor of cardiovascular mortality (27). Apparently, AUC resultant from elevated WHtR as the risk factor may have had a greater performance than elevated WC, which is more commonly used to assess central adiposity. This finding is in agreement with a small study of 81 young adults from UK in which the best predictor of VAT mass and percentage of FM was the WHtR, suggesting that this could be used as a proxy of central adiposity in both sexes (28).

Our regression models suggest that VAT mass and volume may be early markers of cardiometabolic risk even in a sample of young healthy women. After adjustments, the third tertile of VAT remained significantly associated with recognized anthropometric and biochemical cardiometabolic risk factors, but not with diastolic blood pressure. An American study of 723 adults aged 19-47 years (70% Caucasians), using DXA Lunar Prodigy® and enCore software, investigated the linearity of VAT accumulation with the total adiposity and the existence of a sex-related threshold in total fat to identify cardiometabolic risk assessed by hyperglycemic euglycemic clamp (29). Women with mean age of 34 years and BMI of 29.5 kg/m^2 had a mean VAT mass of 600 g, i.e., much higher mean value than found in our study and in European studies (25,26). Increases in VAT were associated with negative effect on insulin sensitivity, reinforcing its role for cardiovascular risk. The statistical significance loss of VAT and blood pressure following adjustments for age or BW in isolation could be attributed to the aging

impact in body fat distribution (30) and to the influence of a low BW in hemodynamics as previously reported (31).

Our study has the limitations of the cross-sectional design that impede to infer causality and sample size. The sample did not represent the female Brazilian young population considering that was composed of highly educated White women. However, data obtained in a high-quality equipment with precise software, from a healthy homogeneous sample, may be an important initial step to define reference values of DXA-determined VAT mass and volume.

In conclusion, DXA-determined values of 221.0 g and 231.8 cm^3 for VAT mass and volume, respectively, should be useful to identify the cardiometabolic risk profile in Brazilian healthy young women. These cutoffs seem able to disclose mild visceral fat accumulation, prior the deterioration of glucose and lipid metabolism, reinforcing the role of VAT as an early cardiometabolic risk marker. Also, the utility of anthropometry for the identification of increased VAT was confirmed, particularly in epidemiological studies. Prospective data are needed to confirm the DXA-VAT ability to detect young at-risk individuals.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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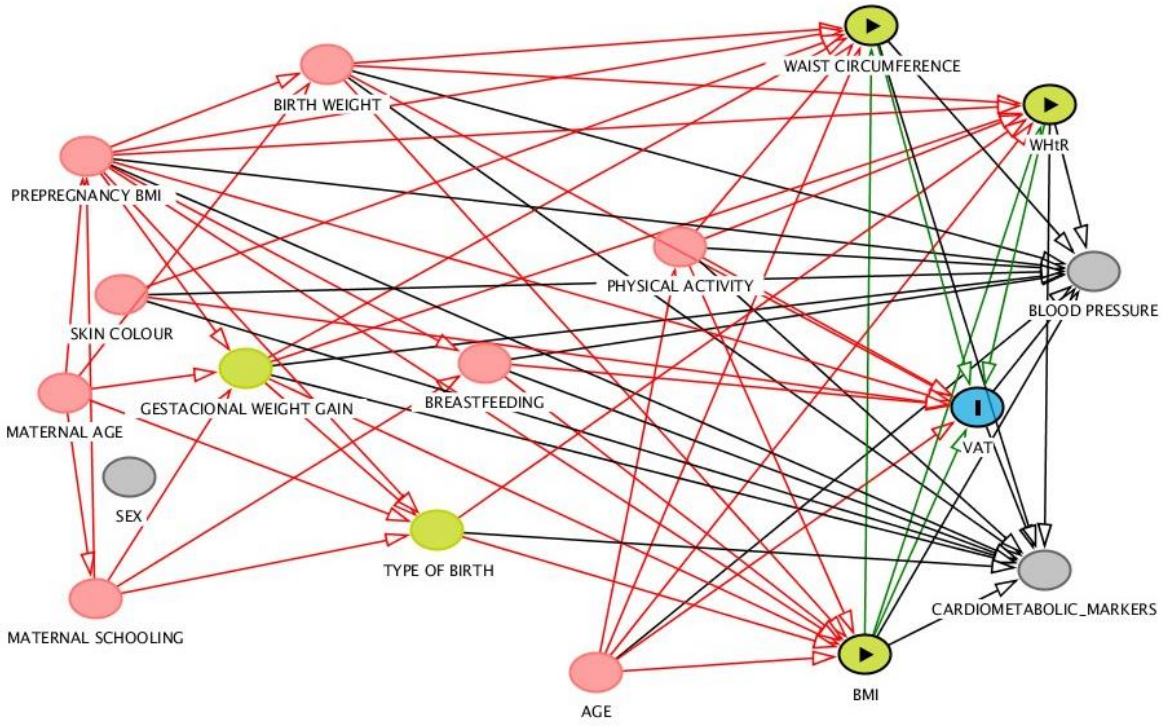

Figure 1. Directed Acyclic Graphs built for estimating the total effect of BMI, waist circumference and WHtR in VAT mass. The minimal sufficient adjustment set included maternal pre-pregnancy BMI, birth weight, breastfeeding, age, skin colour and physical activity.

Table 1. Early life, clinical and biochemical characteristics of participants of study.

	Entire sample	**Sub-sample**	
	n = 201	**n = 148**	**p**
Maternal pre-pregnancy BMI (kg/m^2)	22.5 ± 3.3	22.1 ±3.5	0.757
Birth weight (g)	3,199 ± 424	3,185 ±422	0.501
Percent of breastfeeding[#]	93.7	92.3	0.150
Percent of white skin colour[#]	72.4	69.9	0.192
Percent of physically active [#]	55.7	54.8	0.186
Age (years)*	23.0 (20.0-28.0)	22.0 (20.0-28.0)	0.847
Weight (kg)*	58.9 (54.0-68.2)	58.9 (53.5-69.3)	0.955
Height (m)	1.6 ± 0.6	1.6 ± 0.1	0.072
Body mass index (kg/m^2)	22.9 ± 2.9	23.0 ± 3.0	0.417
Waist circumference (cm)	77.1 ± 8.3	77.7 ± 8.6	0.125
WHtR*	0.5 (0.4-0.6)	0.5 (0.4-0.5)	0.080
Systolic blood pressure (mmHg)	-	107.5 ± 10.6	-
Diastolic blood pressure (mmHg)	-	71.3 ± 8.4	-
Fasting glucose (mg/dL)	-	83.1 ± 9.3	-
Total cholesterol (mg/dL)	-	172.2 ± 34.6	-
LDL-cholesterol (mg/dL)	-	98.4 ± 29.1	-
Non HDL-cholesterol (mg/dL)	-	117.3 ± 32.0	-
HDL-cholesterol (mg/dL)	-	55.0 ± 12.6	-
Triglycerides (mg/dL)	-	92.5 ± 47.8	-
Insulin (μUI/L)	-	9.5 ± 4.3	-
HOMA-IR	-	2.0 ± 0.9	-
TyG index	-	8.1 ± 0.5	-
TyG-BMI index	-	186.6 ± 28.4	-
TyG-WC index	-	630.5 ± 85.2	-
Total body fat (kg)*	19.5 (15.8-24.7)	19.5 (16.5-25.4)	0.271
Fat mass index (kg/m^2)*	7.4 (6.1-9.5)	7.4 (6.1-9.8)	0.181
Android fat (%)	31.7 ± 10.7	32.5 ± 11.1	0.064
Gynoid fat (%)	39.8 ± 6.6	40.4 ± 6.2	0.057
A/G fat ratio	0.8 ± 0.2	0.8 ± 0.2	0.254
VAT mass (g)*	103.0 (36.0-274.0)	108.5 (33.5-333.5)	0.287
VAT volume (cm^3)*	108.0 (38.0-289.0)	112.0 (35.5-345.5)	0.333

Data expressed as means ± standard deviation or medians and interquartile range. * Mann-Whitney test was used. Physically active ≥ 150 min/week.
WHtR: waist-to-height ratio; A/G fat ratio: android-gynoid fat ratio; VAT: visceral adipose tissue, TyG index: triglycerides-glucose index; TyG-BMI: triglycerides–glucose index divided by BMI; TyG-WC: triglycerides–glucose index divided by WC. [#] Data expressed as number (%).

Table 2. Descriptive analyses of outcome variables (visceral adipose tissue – VAT mass in grams and volume in cm^3) for the entire sample and sub-sample.

	Entire sample N = 201	**Sub-sample N = 148**	**p-value**
VAT mass (g)			
Mean (± SD)	221.0 ± 306.1	250.6 ± 342.7	
Median (IQR)	103.0 (36.0-274.0)	108.5 (32.5-333.5)	0.728
Percentiles of VAT mass			
• P10	11.0	11.0	
• P25	36.0	32.5	
• P50	103.0	108.5	
• P75	274.0	333.5	
• P90	577.0	653.0	
VAT volume (cm^3)			
Mean (± SD)	231.8 ± 323.8	262.3 ± 362.8	
Median (IQR)	108.0 (38.0-289.0)	112.0 (35.5-345.5)	0.805
Percentiles of VAT volume			
• P10	12.0	12.0	
• P25	38.0	35.5	
• P50	108.0	112.0	
• P75	289.0	345.5	
• P90	611.0	692.0	

SD: standard deviation; IQR: interquartile range; P: percentile

Table 3. Area under the curve (95% confidence intervals) and cutoff points for visceral adipose tissue (VAT) mass and volume for the entire sample (n = 201) taking cardiometabolic risk factors as outcomes.

	Area under the curve (95% CI)	Cutoff	Sensitivity (%)	Specificity (%)
• ***VAT mass (g)***				
Body mass index ≥ 25 kg/m^2	0.891 (0.836;0.946)	176.0	85.3	85.0
WC ≥ 80 cm	0.835 (0.765;0.905)	116.0	84.6	70.6
WHtR ≥ 0.5	0.851 (0.784;0.918)	176.0	81.8	80.8
Systolic BP ≥ 130 mmHg	0.485 (0.400;0.570)	105.0	51.5	51.9
Diastolic BP ≥ 85 mmHg	0.529 (0.445;0.612)	106.0	50.8	52.1
Plasma glucose ≥ 100 mg/dL	0.472 (0.393;0.560)	101.0	47.4	47.2
HOMA-IR ≥ 2.0	0.603 (0.524;0.683)	99.0	58.5	57.8
Triglycerides ≥ 150 mg/dL	0.537 (0.454;0.619)	119.0	60.3	59.4
HDL-cholesterol < 50 mg/dL	0.612 (0.518;0.706)	114.0	45.6	51.4
TyG ≥ 8.4	0.596 (0.499;0.694)	116.0	58.0	57.8
TyG-BMI ≥ 198	0.823 (0.733;0.912)	142.0	80.0	78.2
TyG-WC ≥ 658	0.864 (0.796;0.931)	154.0	80.0	80.9
• ***VAT volume (cm^3)***				
Body mass index ≥ 25.0 kg/m^2	0.882 (0.824;0.939)	164.0	83.6	82.1
WHtR ≥ 0.5	0.841 (0.772;0.909)	178.0	80.0	79.5
WC ≥ 80 cm	0.826 (0.755;0.897)	141.0	78.5	77.9
Systolic BP ≥ 130 mmHg	0.489 (0.404;0.574)	92.0	45.6	45.1
Diastolic BP ≥ 85 mmHg	0.519 (0.436;0.603)	107.0	49.2	48.6
Triglycerides ≥ 150 mg/dL	0.481 (0.397;0.565)	109.0	51.5	51.1
HDL-cholesterol < 50 mg/dL	0.599 (0.518;0.679)	123.0	60.3	58.7
Plasma glucose ≥ 100 mg/dL	0.542 (0.459;0.624)	105.0	47.4	47.2
HOMA-IR ≥ 2.0	0.601 (0.507;0.695)	105.0	57.6	57.8
TyG ≥ 8.4	0.586 (0.486;0.686)	126.0	57.8	57.1
TyG-BMI ≥ 198	0.807 (0.716;0.898)	150.0	77.1	78.2
TyG-WC ≥ 658	0.851 (0.778;0.924)	150.0	80.0	78.7

WHtR: waist-to-height ratio; WC: waist circumference; BP: blood pressure; HOMA-IR: homeostasis model assessment – insulin resistance; TyG: triglycerides–glucose index;TyG-BMI: triglycerides–glucose index divided by BMI; TyG-WC: triglycerides–glucose index divided by WC.

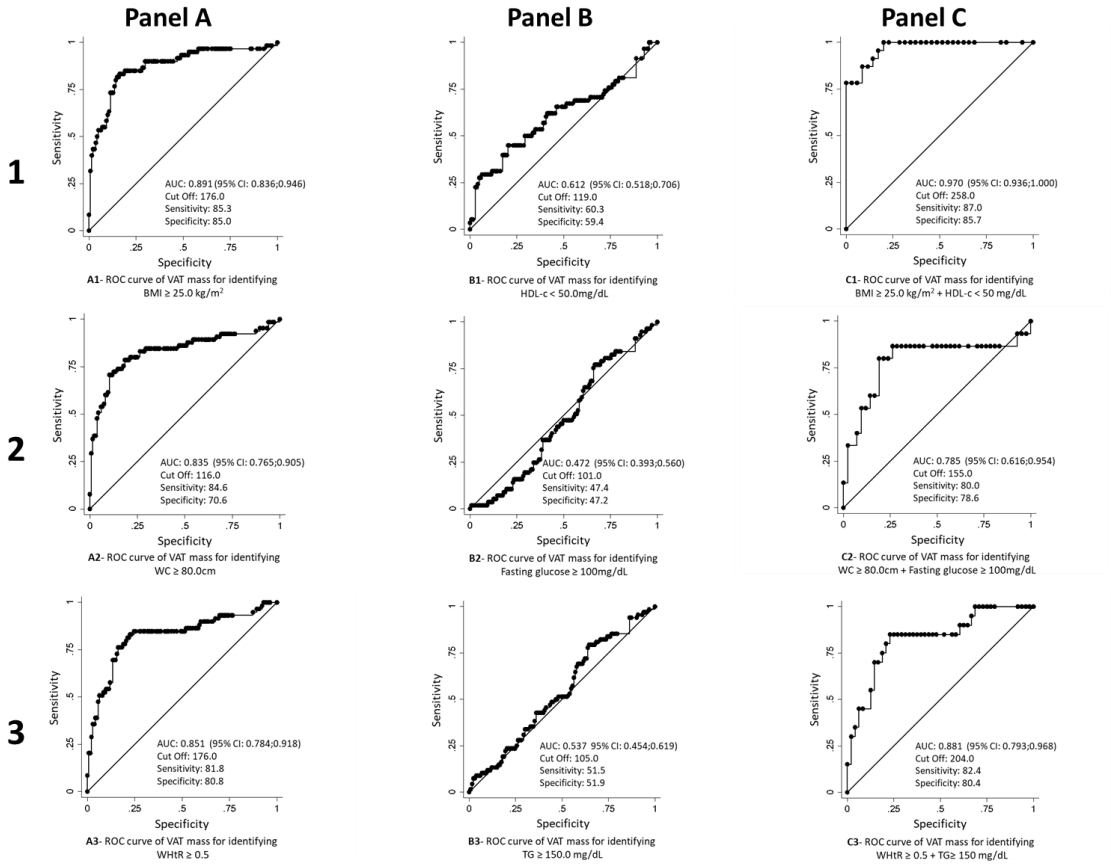

Figure 2. Receiver operating characteristics of VAT mass for identifying abnormalities in cardiometabolic risk factors based on: Panel A: anthropometric data only (A1: BMI ≥ 25 kg/m^2, A2: WC ≥ 80 cm, A3: WHtR ≥ 0.5); Panel B: laboratory data only (B1: HDL-c < 50 mg/dL, B2: FG ≥ 100 mg/dL, B3: TG ≥ 150 mg/dL); Panel C: combined data (C1: BMI ≥ 25 kg/m^2 + HDL-c < 50 mg/dL, C2: WC ≥ 80 cm + FG ≥ 100 mg/dL, C3: WHtR + TG ≥ 150 mg/dL).

BMI: body mass index; WC: waist circumference; WHtR: waist-to-height ratio; HDL-c: HDL-cholesterol; FG: fasting glucose; TG: triglycerides.

Table 4. Prevalence ratios (95% confidence intervals – CI) of cardiometabolic risk factors associated with visceral adipose tissue (VAT) mass and volume at the third tertiles, obtained from 201 young women, participants of the NutriHS.

	Crude			**Adjusted model**		
	Prevalence ratio	95% CI	p-value	Prevalence ratio	95% CI	p-value
• ***VAT mass (g)***						
Body mass index	1.02	1.008-1.041	0.003	1.10	1.060-1.136	<0.001
Waist circumference	1.02	1.015-1.034	<0.001	1.04	1.025-1.049	<0.001
WHtR	1.64	1.376-1.943	<0.001	1.59	1.307-1.931	<0.001
Diastolic blood pressure	1.02	1.008-1.033	0.001	1.02	0.999-1.035	0.072
HDL-cholesterol	1.00	0.988-1.006	0.538	1.00	0.989-1.009	0.842
Triglycerides	1.00	1.001-1.003	<0.001	1.00	1.002-1.006	<0.001
HOMA-IR	1.23	1.119-1.347	<0.001	1.24	1.090-1.420	0.001
TyG	1.36	1.114-1.665	0.003	1.52	1.166-1.970	0.002
TyG-BMI	1.01	1.007-1.014	<0.001	1.01	1.006-1.016	<0.001
TyG-WC	1.00	1.002-1.004	<0.001	1.00	1.002-1.005	<0.001
• ***VAT volume (cm^3)***						
Body mass index	1.02	1.008-1.040	0.004	1.09	1.049-1.127	<0.001
Waist circumference	1.02	1.014-1.033	<0.001	1.03	1.023-1.046	<0.001
WHtR	1.58	1.330-1.886	<0.001	1.51	1.241-1.842	<0.001
Diastolic blood pressure	1.07	1.003-1.030	0.016	1.01	0.990-1.030	0.346
HDL-cholesterol	1.00	0.988-1.006	0.528	1.00	0.989-1.009	0.878
Triglycerides	1.00	1.001-1.003	<0.001	1.00	1.002-1.006	0.001
HOMA-IR	1.21	1.105-1.328	<0.001	1.21	1.059-1.375	0.005
TyG	1.35	1.101-1.646	0.004	1.49	1.143-1.949	0.003
TyG-BMI	1.01	1.006-1.014	<0.001	1.01	1.005-1.015	<0.001
TyG-WC	1.00	1.002-1.004	<0.001	1.00	1.002-1.005	<0.001

Poisson regression used.

Adjusted model: each independent variable entered into separate models that were adjusted for maternal pre-pregnancy BMI, birth weight, breastfeeding, age skin colour and physical activity according to directed acyclic graph (DAG).

WHtR: waist-to-height ratio; TyG: triglyceride–glucose index;TyG-BMI: triglyceride–glucose index divided by body mass index; TyG-WC: triglyceride–glucose index divided by waist circumference.

$p < 0.05$ was considered significant.

5.2. PAPER 2

Birth weight is associated with dual energy X-ray absorptiometry-determined muscle-bone unit in young healthy women from the Nutritionists' Health Study

Running title: Birth weight and muscle-bone unit

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ABSTRACT

BACKGROUND: Muscle and bone have been considered a functional unit that grows together early in life, deteriorates with aging and can cause osteosarcopenia. Due to its importance in public health, detecting risk factors in early life is desirable. This study examined whether birth weight (BW) was associated with muscle-bone unit using dual energy X-ray absorptiometry (DXA) parameters in young women from the NutriHS, a cohort study of undergraduates and Nutrition graduates.

METHODS: This cross-sectional analysis included 201 young healthy women who answered early life events- questionnaire, and had anthropometric, muscle strength and performance, DXA-determined body composition and bone densitometry (iDXA Lunar®, EnCore Software) data and blood sample collected. Appendicular skeletal muscle mass index (ASMI) was calculated. BW was categorized in quartiles (BWq) and variables of interest compared by ANOVA. Associations of BWq (exposure) with calf circumference (CC), handgrip, muscle performance tests, ASMI, bone mineral density and content (BMD and BMC), and plasma glucose, lipids, insulin and 25-hydroxyvitamin D (outcomes) were performed using multiple linear regression and directed acyclic graph-recommended adjustments.

RESULTS: Mean values of age, BMI and BW were 23.0 years (20.0-28.0), 22.9±2.9 kg/m^2 and 3,199±424 g, respectively. Comparing variables across BWq, significant differences in CC, HG, ASMI and total body BMC were detected. Regression models adjusted for confounders showed associations of BWq with CC (β=0.72, p=0.005), handgrip (β=1.53, p=0.001) and ASMI (β=0.16, p=0.022). BWq were also associated with total body BMC (β=64.8, p=0.005), total femur BMC (β=0.70, p=0.041), total body BMD (β=0.02, p=0.043) and lumbar spine BMD (β=0.03, p=0.028).

CONCLUSIONS: We conclude that BW is associated with muscle-bone unit using DXA-parameters in Brazilian young healthy women from the NutriHS, suggesting a role for intrauterine environment for musculoskeletal health.

Key words: muscle mass; bone mass; DXA; osteosarcopenia; birth weight; DOHaD.

BACKGROUND

Recently, muscle and bone have been considered a functional unit (1). There is evidence that both tissues start growing early in life, parallelly until reaching their peaks around age 25 years. The integrity of the biochemical and biomechanical systems in such musculoskeletal unit is essential to guarantee a healthy aging. Muscle and bone masses are maintained during adulthood, and deterioration rates depend on several genetic, epigenetic and environmental factors (1,2). Co-existence of osteoporosis and sarcopenia is named osteosarcopenia (3) that is a chronic condition, typically manifested in frail elderly population and associated with disability and mortality (4).

Osteoporosis and sarcopenia share common risk factors and pathophysiological pathways (5). Since known risk factors do not fully explain the occurrence of these diseases (1), the search for new causal factors is needed, such as those present in early stages of life. According to the developmental origins of health and disease theory (DOHaD), insults occurring in the early stages of development can generate epigenetic changes and predispose to non-communicable chronic diseases in adulthood (6-8). Birth weight (BW) has been considered a major proxy of the quality of the intrauterine life (7). Insults in the intrauterine environment are known to trigger epigenetic modifications that alter gene expression and permanently set pathways linked to chronic diseases (10). The intergenerational epidemiology investigates how epigenetic modifications can be transmitted to following generations (9).

Studies have examined whether BW would be associated with muscle performance in midlife and advanced age. Positive association of BW with grip strength was found in middle-aged adults (10), as well as with sarcopenia in elders (11). Also, in a birth cohort conducted in Brazil, BW was associated with grip strength in adulthood (12). Additionally, the same group reported that BW predicted bone mass assessed by densitometry in adults at age 18 years (13). Such association was confirmed in young women (14). These findings have indicated that changes in muscle and bone compartments should occur as a continuous process during the life course. Improving detection of risk factors for muscle-bone unit before functional deterioration could prevent disabilities later in life. Dual energy X-ray absorptiometry

(DXA) is a non-invasive technique that provide accurate measurements of both compartments (15).

As far as we know, in healthy young adults, no study has explored association of early life events with the status of muscle and bone tissues concomitantly for early prevention of osteosarcopenia. Our group has conducted the Nutritionists' Health Study (NutriHS), whose design allowed testing associations of self-reported data from early life with muscle and bone outcomes before advanced age. Therefore, we had the opportunity to examine whether BW was associated with the muscle-bone unit using DXA-determined parameters in young healthy women from the NutriHS.

MATERIALS AND METHODS

- Study design, setting, and participants

Current analyses were performed using baseline data of the NutriHS, launched in 2014. Recruitment and data collection occurred from 2015 to 2018. NutriHS includes undergraduates and graduates from Nutrition courses in São Paulo State, Brazil. It was approved by institutional ethics committee and all the participants signed the informed consent electronically, using the NutriHS website (www.fsp.usp.br/nutrihs). Details on its purposes and methodological aspects were previously reported (16).

The present study has a mixed design. Once our main exposure variable, the BW, was recovered from the questionnaire, it was considered a historical cohort. Also, cross-sectional analyses on the associations with outcomes were performed.

Participants answered online questionnaires regarding sociodemographic data, family history, early life events, lifestyle, current diet and clinical data (17, 18). Diet was assessed using validated food frequency questionnaire (16) and physical activity using the short form of the international physical activity questionnaire (18). An e-mail was sent to schedule a visit to the health care center of the School of Public Health of the University of São Paulo. They were submitted to clinical examination and had biological samples collected. In a second visit, muscle tests were performed, as well as bone densitometry and body composition.

A convenience sample of the first 201 participants of NutriHS who met eligibility criteria was taken. Inclusion criteria were: women aged 20 to 45 years, with regular menstruation, no past or current history of weight changes > 5% of total body weight in the last year, malignancy, use of medications other than contraceptives, elevated blood pressure, and disturbances of glucose and lipid metabolism. Exclusion criteria were to be pregnant and anthropometric measures exceeding the limits of the densitometer (1.87 m and/or 201 kg, respectively). All participants answered the questionnaires and were submitted to anthropometry, bone densitometry and body composition by DXA. Information about their BW was missing for 31 participants. A random sub-sample of 148 participants was submitted to blood pressure measurement and laboratory examination.

- Variables

BW was our exposure variable. It was obtained as continuous variables (in grams) and after categorized in quartile. Outcomes were calf circumference (CC), muscle strength and performance, muscle mass and bone mineral density and content (BMD and BMC) obtained by DXA. Other variables of interest were maternal information, other early life events, and biochemical results.

- *Clinical measurements*

Weight was measured with digital scale (Filizola®, São Paulo, Brazil) to the nearst 0.1 kg, height with a fixed stadiometer with 0.1 cm precision, and BMI was calculated. CC was assessed at the mid-calf point, with the participant in the orthostatic position. Blood pressure was taken three times, using an automatic device (Omron model HEM-712C®, Omron Health Care Inc, USA), with an appropriate cuff for the circumference of the participant's arm, in sitting position after 5-minute rest. The values considered for analyses were the mean of the last two measurements.

- *Muscle mass, strength and performance*

Calf circumference was taken as a clinical measure of muscle mass and was obtained at the mid-calf point. Muscle strength was assessed by the palmar grip strength (19), using a manual dynamometer (Jamar®). Participants sit with the spine erect, maintaining the bend angle of the knee at 90° and feet resting on the floor, the shoulder positioned in adduction and neutral rotation, and the elbow flexed at 90°. The arm was held suspended in the air with the hand positioned on the dynamometer,

which was adjusted in the second position. Measurements were made in duplicate in the dominant limb, respecting the interval of one minute to avoid muscular fatigue, and it is considered the mean value. The participant was instructed to tighten the dynamometer with the highest possible force for three seconds and then release; the result was then read out. A first test was performed just for learning purposes. The chair-stand test was used to evaluate strength of legs and the gait speed test muscle performance. For the chair-stand test, a straight-backed chair with a solid seat was used. Participant was asked to sit with arms crossed over the chest, and the time to get up and sit as fast as possible five times was measured (20). In gait speed test, the participant was instructed to walk at her normal pace on a 4-meter course; time was checked, and results provided in meters per second (21).

- *DXA-derived measurements*

DXA was used to measure: a) bone mineral density and content of total body (TB), lumbar spine (LS), femoral neck (FN) and total femur (TF), and b) lean mass of arms and legs, whose sum allows obtaining the appendicular skeletal muscle mass (ASM). Then, the appendicular skeletal muscle mass index (ASMI) was calculated dividing the ASM by squared height in meters. Bone densitometry and body composition scans were performed and analysed by the same researcher, certified by the Brazilian Association of Bone and Osteometabolism (ABRASSO), in the GE Lunar® iDXA with EnCore sotware (Madison, WI, USA). Regions of interest (ROIs) were determined manually according to the manufacturer's specifications; densitometer calibrations were performed routinely. Precision values and least significant change (LSC) were calculated according to the International Society for Clinical Densitometry (https://www.iscd.org/) after three successive measurements in fifteen women. The LSC of bone densitometry scans were: - LS BMD: 0.027 g/cm^2; - TF BMD: 0.017 g/cm^2; and - FN BMD: 0.056 g/cm^2. The precision values of body composition were: - TB BMD: 0.009 g/cm^2; - total lean mass: 275.0 g; and - ASM: 350.0 g.

Biochemical analyses

Fasting plasma glucose, total cholesterol, LDL-cholesterol (LDL-c), HDL-cholesterol (HDL-c), triglycerides, insulin, and 25-hydroxyvitamin D were determined. Plasma glucose was obtained by the glucose oxidase method and insulin by

immunofluorimetric assay, based on monoclonal antibody (Monobind, Inc., Lake Forest, CA, USA). Insulin resistance was estimated by the HOMA-IR index. The lipid profile was analyzed by colorimetric and enzymatic methods and LDL-c was calculated by the Friedwald equation. Vitamin D levels were measured by electrochemiluminescence assay on Elecsys analyzers and Cobas modular platforms (Roche® Diagnostics, Indianapolis, USA).

- Statistical analysis

Data were presented as mean ± standard deviation (SD). The normality of the variables was verified by the Kolmogorov-Smirnov test. The correlation between variables was tested using Pearson coefficient. Variables of interest were compared by ANOVA and post hoc Bonferroni among the quartiles of BW. Independent associations of BW quartiles (exposure) with CC, strength assessed by handgrip, muscle performance tests, ASM, ASMI, BMD, BMC and plasma glucose, lipids, and 25-hydroxyvitamin D levels (outcomes) were tested using multiple linear regression and Directed Acyclic Graph-recommended (DAG) minimal sufficient adjustments (22) (www.dagitty.net) (figure 1). STATA 13.1® statistical package was used and $p < 0.05$ was considered significant.

RESULTS

For the entire sample and the sub-sample, mean reported maternal prepregnancy BMI was within the normal range and participants'mothers had adequate weight gain during pregnancy. Our sample and sub-sample were composed of young people with normal BW and adequate anthropometry measurements (Table 1); approximately 72% reported white skin color. The sub-sample had mean normal blood pressure levels and laboratory variables, including 25-hydroxyvitamin D.

The majority of participants' mother had at least five years of education and did not smoke during pregnancy (Table 2). Cesarean delivery was the most common type of delivery and most participants had been breastfed for at least 6 months. The majority was non-smokers and non- alcohol consumers, and practice regular physical activity, and almost two thirds were using contraceptives.

BW was positively correlated to bone (TB BMC: r = 0.20, p = 0.010; TF BMC: r = 0.17, p = 0.027) and muscle mass and strength (CC: r = 0.24, p = 0.001; ASM: r = 0.24, p = 0.002; ASMI: r = 0.20, p = 0.011; handgrip-determined strength: r = 0.17, p = 0.023; Figure 2), but not to muscle performance. Strong correlations were also detected between CC and ASM (r = 0.68, p < 0.001) and ASMI (r = 0.77, p < 0.001).

Mean values of muscle and bone compartments variables (n = 201) across BW quartiles (n = 170) were in Table 3. Differences in CC (34.7 ± 3.2 *versus* 36.6 ± 3.2 cm, p = 0.029), ASM (15.5 ± 3.0 *versus* 17.7 ± 3.7 kg, p = 0.015), ASMI (6.0 ± 0.8 *versus* 6.6 ± 1.0 kg/m^2, p = 0.034), muscle strength (23.4 ± 4.8 *versus* 26.6 ± 4.8 kg, p = 0.021) and TB BMC (2,210 ± 272 *versus* 2,388 ± 293 g, p = 0.020) between the first and last BW quartiles, respectively, were detected.

Linear regression models showed that BW quartiles were independently associated with CC, ASM, ASMI and muscle strength after adjustments for maternal gestational weight gain, maternal pre-pregnancy BMI, breastfeeding, skin color and type of birth (Table 4). BW quartiles, adjusted for the same covariables, were also associated with bone parameters, TB BMD, LS BMD, TB BMC and TF BMC.

DISCUSSION

For the best of our knowledge, we are the first to investigate the association of the musculoskeletal unit functionality with BW – taken as a proxy of intrauterine environment – early in adulthood, without any manifestation of osteosarcopenia. The finding of independent associations with both, muscle (mass and strength), and bone (mineral density and content) parameters in young healthy women reinforce that the impact of BW might be early perceived. Additionally, our study was useful to provide DXA results of muscle and bone compartments, as well as parameters of strength and muscle performance, not previously described for a healthy stratus of the Brazilian women.

Some investigators raised the possibility of an influence of BW on muscular strength in midlife (10), and later they reported an association with sarcopenia in elderly (11). The hypothesis that sarcopenia could have its origins in early life was reinforced in a systematic review (10) and further confirmed in birth cohorts (12, 23). Our present findings support the idea that consequences of intrauterine insults should

manifest even in apparently healthy individuals, when preventive measures could be employed. Since dynapenia is related to several adverse health outcomes in later life (24), it is relevant to start prevention as early as possible in terms of public health. Results of handgrip of the participants of our study (24.5 ± 5.0 kg) were within the normal range, but lower than those observed in a Brazilian cohort of 3,470 adults aged 30 years (29.7 ± 5.4 kg) (12) and in an English study with women aged 20-40 years (32.2 ± 5.9 kg) (23). A possible explanation for lower muscle strength of our participants could be lower percentage of weight excess (35 versus 51%) and lean mass (17 versus 39 kg) than in the Brazilian cohort, respectively.

Using DXA in the evaluation of 229 non-Hispanic Americans, aged 18-40 years, Baumgartner et al. proposed the ASMI and defined as mean values ≤ 8.60 kg/m^2 and ≤ 7.30 kg/m^2 for men and women, respectively (25); lower values (≤ 7.26 kg/m2 and ≤ 5.45 kg/m^2) were established for of sarcopenia diagnosis (21). However, ethnic differences in lean mass have been reported (26,27). Mexicans had lean mass lower than White non-Hispanic Americans (26), as well as non-Black Brazilian healthy women compared to Americans from distinct ethnic groups (27). Comparisons at similar age groups, indicate that participants of our study had higher mean values of ASM and ASMI than Mexicans and Brazilians, but lower than a young American population (25-27). We call attention to the importance of population-specific cutoffs for these DXA indices due to their impact in sarcopenia diagnosis.

Recent consensus of the European Working Group on Sarcopenia in Older People recognized that muscle strength is better than mass in predicting adverse outcomes (28). Although strength overtakes the role of low muscle mass, detections of low muscle quality and quantity are important to confirm the sarcopenia diagnosis and physical performance and to define the sarcopenia severity. Our study provides subsidies to suppose that BW could be related to both muscle strength and mass. Since it was recognized that the development of sarcopenia begins early in life, a low BW could indicate a need to maximize muscle in youth and young adulthood, in order to maintain muscle in middle age and minimize loss in older age (28). This new paradigm reinforces the importance of studies such ours. As a matter of fact, we used tools recommended by the European sarcopenia consensus, i.e. handgrip and chair-stand tests, to assess strength of leg muscles. DXA has been the most widely used method to determine muscle quantity non-invasively, while the gait speed indicates

physical performance. However, CC could be used a proxy of muscle mass in older adults when no accurate method is available (28). In our sample, CC was strongly correlated to ASM and ASMI and, as expected, values were greater than those recommended for elderly women from several populations (29, 30).

Considering the new cut-off points for sarcopenia in women (handgrip < 16 kg; chair-stand test > 15 seconds; ASM < 15 kg; ASMI < 6.0 kg/m2; gait speed ≤ 0.8 m/s), participants of our study showed adequate results in all the tests. However, when analyzing the mean values of muscle parameters across BW quartiles, we observed that the mean values of ASM and ASMI in the first quartile were very close to the minimal cut-off recommended. The latter finding deserves attention considering that they just reach their muscle mass peak (2). Similar to the low BW-conferred risk of other non-communicable chronic diseases (31, 32), our finding suggests that low BW could be also a predictor of lower muscle mass in adulthood and, perhaps, of sarcopenia in advanced age. The follow-up of the NutriHS could explore such hypothesis.

In addition to recognized factors with contribute to accumulation of bone mass during childhood and adolescence – heredity, gender, life habits, endocrinopathies, medications – our findings support an association of BW and weight in infancy with adult bone mass, in agreement with previous studies (13, 33). A systematic review and meta-analysis demonstrated a positive association between BW and bone mass among children although weak in adults (34), concluding that BW may favor bone health in later life. On the other hand, a European birth cohort verified that prenatal growth had no significant impact in TB and LS BMD in early adulthood, but the weight gain during childhood (35).

Our results are consistent since positive associations of BW quartiles occurred with several bone parameters (TB BMD, LS BMD, TB BMC and TF BMC), after several adjustments (maternal gestational weight gain, maternal pre-pregnancy BMI, breastfeeding, skin color and type of birth). All participants presented regular menstruation, and bone parameters were not associated with menarche age, physical activity, smoking, alcohol intake, contraceptive or steroid use or vitamin D levels. Interestingly, positive correlations between the muscle and bone parameters suggested a crosstalk between the tissues and reinforcing the importance of the concomitant evaluation of these compartments.

Our findings in young healthy women from the NutriHS are in line with the importance of the first 1,000 days of life for musculoskeletal health. A healthy intrauterine environment, as well as the development in infancy, should enable adequate muscle and bone mass peaks in adulthood reducing the risk of osteosarcopenia in elderly age.

Our study has the limitation of its cross-sectional design and a relatively small sample size. Self-reported BW data are commonly used and were validated in previous studies. Our selected sample impedes extrapolation of the results for Brazilian young female population. However, data from a healthy and homogeneous sample (highly educated White women) could represent also a strength of our study, since we describe an adequate musculoskeletal profile that may be useful for future comparisons. An important strength was DXA assessment of musculoskeletal unit functionality, i.e. muscle and bone compartments concomitantly.

We conclude that BW is associated with muscle-bone unit using DXA-parameters in Brazilian young healthy women from the NutriHS, suggesting a role for intrauterine environment for musculoskeletal health. Prospective studies are necessary to investigate the ability of BW to predict osteosarcopenia risk in the long-term.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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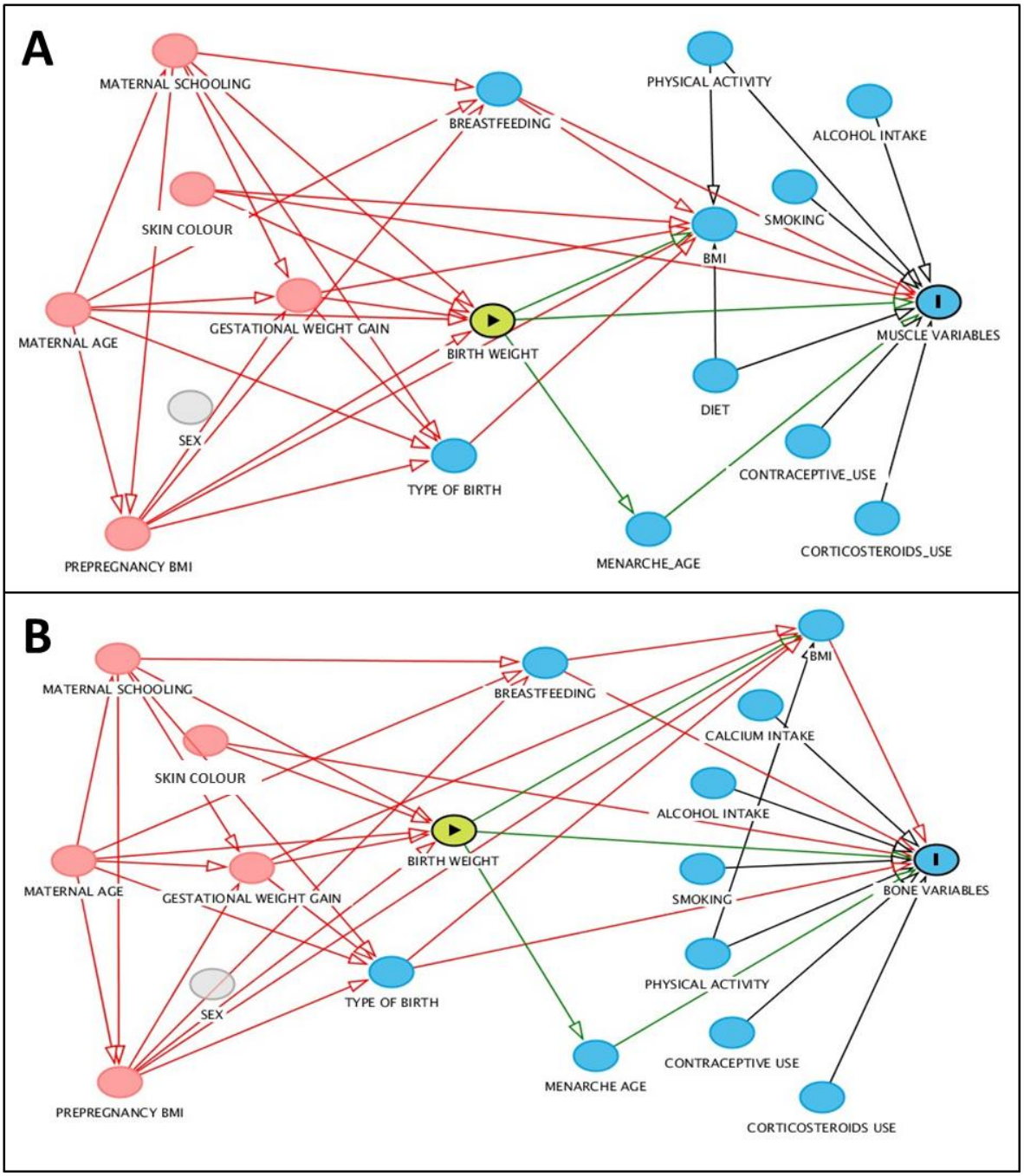

Figure 1. Directed Acyclic Graphs built for obtaining minimal sufficient adjustments for the total effect of birth weight in: **A-** Muscle variables. **B-** Bone variables. For both, the minimal sufficient adjustment set included maternal gestational weight gain, maternal pre-pregnancy body mass index, breastfeeding, skin colour and type of birth.

Table 1. Early life, clinical and biochemical characteristics of participants of study.

	Entire sample n = 201	**Sub-sample n = 148**	**p-value**
Maternal pre-pregnancy BMI (kg/m^2)	22.5 (3.3)	22.1 (3.5)	0.757
Maternal pregnancy weight gain (kg)	12.8 (9.0-15.0)	12.8 (9.0-15.0)	0.890
Age (years)*	23.0 (20.0-28.0)	22.0 (20.0-28.0)	0.847
Birth weight (g)$^{\$}$	3,199 (424)	3,185 (422)	0.501
Menarche age (years)	12.1 (11.0-13.0)	12.0 (11.0-13.0)	0.962
Body mass index (kg/m^2)	22.9 (2.9)	23.0 (3.0)	0.417
Waist circumference (cm)	77.1 (8.3)	77.7 (8.6)	0.125
Systolic blood pressure (mmHg)	-	107.5 (10.5)	-
Diastolic blood pressure (mmHg)	-	71.3 (8.4)	-
Plasma glucose (mg/dL)	-	83.1 (9.3)	-
Total cholesterol (mg/dL)	-	172.2 (34.6)	-
LDL-cholesterol (mg/dL)	-	98.4 (29.1)	-
HDL-cholesterol (mg/dL)	-	54.9 (12.5)	-
Triglycerides (mg/dL)	-	92.8 (47.8)	-
Insulin (μUI/L)	-	9.5 (4.2)	-
HOMA-IR	-	2.0 (0.9)	-
25-hydroxyvitamin D (ng/mL)	-	26.2 (11.1)	-

Data expressed as means and standard deviation or medians and interquartile range.
$^{\$}$ Data available for 170 participants.
* Mann-Whitney test was used.

Table 2. Maternal, neonatal period, and current characteristics of the participants of study.

Years of maternal education	0 to 4	23.9
	5 to 11	45.7
	≥ 12	30.4
Maternal smoking during pregnancy	yes	3.0
	no	97.0
Type of birth	vaginal	48.5
	cesarian section	51.5
Breastfeeding duration	< 6 months	28.6
	6 – 12 months	38.3
	≥ 12 months	33.1
Skin colour	white	72.4
	non white	27.6
Current smoking	yes	8.2
	no	91.8
Current alcohol intake	yes	39.6
	no	60.4
Contraceptive use	yes	74.0
	no	26.0
Physical activity ≥ 150 min/week	yes	55.7
	no	44.3

Data expressed as number (%).

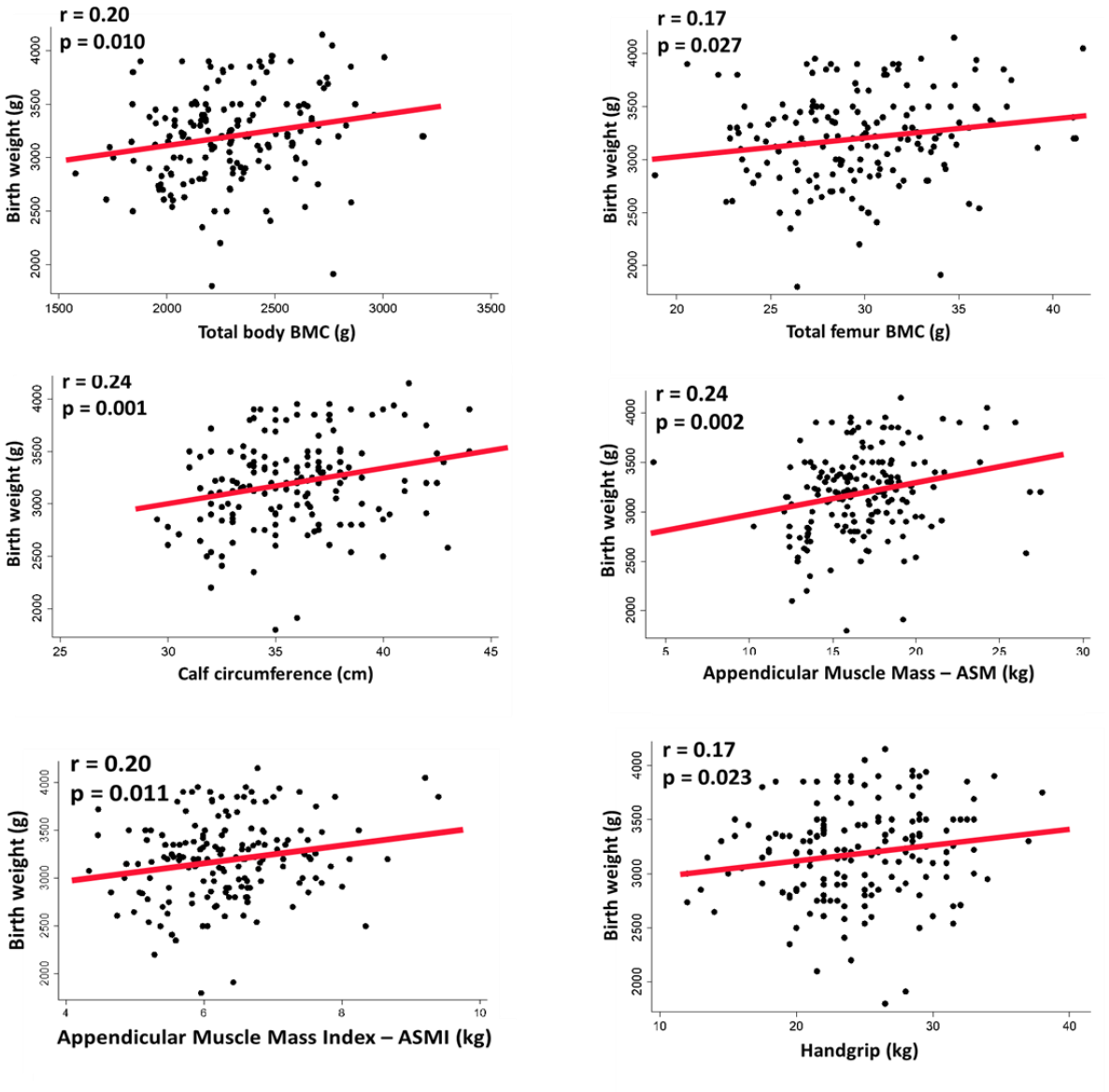

Figure 2. Graphic representation of correlations between birth weight and bone (total body and total femur BMC) and muscle variables (calf circumference, appendicular muscle mass, appendicular muscle mass index and handgrip strength).

Pearson correlation coefficient used.
BMC: bone mineral content.

Table 3. Mean values of muscle and bone variables for the entire sample (n = 201) and for 170 participants stratified in quartiles of birth weight.

	Entire sample	**1st quartile** n = 43 2500–2900 g	**2nd quartile** n = 43 2901-3200 g	**3rd quartile** n = 42 3201-3480 g	**4th quartile** n = 42 3481-4150 g	p-value
Calf circumference (cm)	35.8 (2.9)	34.7 (3.2)	35.9 (2.9)	36.1 (2.6)	36.6 (3.2)	0.027
ASM (kg)	16.9 (3.1)	15.5 (3.0)	17.1 (3.2)	16.7 (2.2)	17.7 (3.7)	0.015
ASMI (kg/m^2)	6.4 (0.9)	6.0 (0.8)	6.4 (1.0)	6.3 (0.8)	6.6 (1.0)	0.034
Handgrip strength (kg)	24.5 (5.0)	23.4 (4.8)	23.9 (5.2)	24.5 (5.0)	26.6 (4.8)	0.021
Gait speed test (m/sec)	1.0 (0.1)	1.0 (0.2)	1.0 (0.1)	1.0 (0.1)	1.0 (0.1)	0.819
Chair-stand test (sec)	10.0 (2.1)	9.6 (1.9)	9.6 (1.9)	10.2 (2.3)	9.8 (2.0)	0.401
TB BMD (g/cm^2)	1.1 (0.10)	1.1 (0.1)	1.1 (0.1)	1.1 (0.1)	1.2 (0.1)	0.278
TB BMC (g)	2,306 (288)	2,210 (272)	2,266 (270)	2,349 (304)	2,388 (293)	0.020
LS BMD (g/cm^2)	1.2 (0.14)	1.2 (0.1)	1.2 (0.1)	1.2 (0.1)	1.2 (0.2)	0.310
LS BMC (g)	55.4 (16.6)	54.7 (14.0)	54.2 (17.4)	55.5 (17.2)	54.1 (18.2)	0.976
TF BMD (g/cm^2)	1.0 (0.13)	1.0 (0.1)	1.0 (0.1)	1.0 (0.2)	1.0 (0.1)	0.662
TF BMC (g)	29.8 (4.17)	28.6 (3.6)	29.6 (3.7)	30.3 (4.6)	30.7 (4.6)	0.117
FN BMD (g/cm^2)	1.0 (0.13)	1.0 (0.1)	1.0 (0.1)	1.0 (0.1)	1.0 (0.1)	0.697
FN BMC (g)	4.6 (0.7)	4.5 (0.6)	4.5 (0.5)	4.8 (0.8)	4.6 (0.8)	0.168

ASM: appendicular skeletal muscle mass; ASMI: appendicular skeletal muscle mass index; BMD: bone mineral density; BMC: bone mineral content; TB: total body; LS: lumbar spine; TF: total femur and FN: femoral neck.

Table 4. Linear regression models of muscle and bone variables associated with birth weight in quartiles, obtained from 170 participants of the NutriHS.

	Crude			Adjusted model		
	β	95% CI	p-value	β	95% CI	p-value
• ***Muscle compartment***						
Calf circumference (cm)	0.63	0.227-1.031	0.007	0.72	0.224-1.209	0.005
ASM (kg)	0.60	0.174-1.035	0.006	0.71	0.231-1.183	0.004
ASMI (kg/m^2)	0.15	0.029-0.268	0.015	0.16	0.024-0.301	0.022
Handgrip strength (kg)	1.06	0.396-1.717	0.002	1.53	0.664-2.396	0.001
Gait speed test (m/sec)	0.01	-0.016-0.027	0.622	0.01	-0.016-0.046	0.348
Chair-stand test (sec)	0.14	0.141-0.418	0.330	1.01	-0.367-0.383	0.966
• ***Bone compartment***						
TB BMD (g/cm^2)	0.01	-0.001-0.025	0.071	0.02	0.001-0.033	0.043
TB BMC (g)	61.79	23.30-100.27	0.002	65.78	19.87-111.69	0.005
LS BMD (g/cm^2)	0.02	-0.001-0.037	0.065	0.03	0.003-0.053	0.028
LS BMC (g)	-0.06	-2.310-2.194	0.960	0.18	-2.118-2.487	0.874
TF BMD (g/cm^2)	0.01	-0.007-0.029	0.216	0.02	-0.005-0.043	0.114
TF BMC (g)	0.70	0.126-1.264	0.017	0.70	0.029-1.377	0.041
FN BMD (g/cm^2)	0.01	-0.010-0.025	0.393	0.01	-0.008-0.038	0.205
FN BMC (g)	0.08	-0.018-0.173	0.111	0.11	-0.008-0.228	0.067

Adjusted for maternal gestational weight gain, maternal pre-pregnancy BMI, breastfeeding, skin colour and type of birth

CI: confidence interval; ASM: appendicular skeletal muscle mass; ASMI: appendicular skeletal muscle mass index; BMD: bone mineral density; BMC: bone mineral content; TB: total body; LS: lumbar spine; TF: total femur and FN: femoral neck.

5.3. PAPER 3

Muscle-bone relationships and associated factors from the life cicle in Brazilian healthy young women from the Nutritionists' Health Study

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ABSTRACT

BACKGROUND: Knowledge of muscle-bone crosstalk has enhanced the importance of concomitant clinical examination of sarcopenia and osteoporosis. Early identification of risk factors throughout life course is desirable. We examined whether parameters of muscle and bone sites were associated and possible predictive factors of them throughout life course.

METHODS: This cross-sectional analysis included 201 healthy women (20-45 years) who answered a questionnaire about early life events and had anthropometric data, muscle strength and performance, dual-energy x-ray absorptiometry-determined body composition and bone densitometry and blood sample collected. Appendicular skeletal muscle mass index (ASMI) was calculated. Multiple linear regression, using the directed acyclic graph-recommended minimal sufficient adjustments, were used to test associations of calf circumference (CC), handgrip (HG), chair-stand test, gait speed and ASMI (exposures) with bone mineral density and content (BMD and BMC) (outcomes). Associations of life course factors with muscle and bone parameters were tested.

RESULTS: More than 70% reported white skin colour, median age was 23.0 years (20.0-28.0), and mean values of BMI, CC, HG and ASMI were 22.9±2.9 kg/m^2, 35.8±2.9 cm, 24.4±5.0 kg and 6.4±0.9 kg/m^2, respectively. In regression models, direct associations were detected: total body BMD with CC [β=0.011; p=0.006], ASMI [β=0.041; p=0.003] and HG [β=0.006; p<0.001]; total body BMC with CC [β=49.41; p<0.001], ASMI [β=204.83; p<0.001] and HG [β=23.34; p<0.001]; femoral neck BMC with CC [β=0.085; p=0.006], ASMI [β=0.411; p<0.001] and HG [β=0.041; p<0.001] and total femur BMC with CC [β=0.589; p=0.001], ASMI [β=2.865; p<0.001] and HG [β=0.271; p<0.001]. Life course factors associated with muscle and bone sites were birth weight, physical activity and smoking. Alcohol uses was associated only with muscle mass.

CONCLUSIONS: Our findings in young adults support the muscle-bone crosstalk and suggest predictive factors (such as birth weight, physical activity, smoking and alcohol use) of muscle and bone parameters. This raises the importance of osteosarcopenia risk assessment early in life. Follow-up of the NutriHS participants could investigated such hypothesis.

KEY WORDS: Muscle-bone crosstalk, osteosarcopenia, DXA, DOHaD

BACKGROUND

Knowledge about the crosstalk between muscle and bone and clinical observation of concomitant occurrence of sarcopenia and osteoporosis has called attention to the importance of considering muscle and bone as a unit. Over the decades, the musculoskeletal unit has been simplified and viewed as just a mechanical structure (1). However, it is a complex system and an adequate understanding of the biochemical signaling, as well its associated risk factors, can favor early diagnosis and new therapeutic strategies. In this sense, the number of publications has increased (2), especially in the basic areas, attempting to explain biochemical and biomechanical mechanisms involved in musculoskeletal interaction (3-7). At the same time, other researchers have explored the relationship between muscles and bones from observations and from knowledge originated from musculoskeletal or neurological disorders (8,9).

The secretory capacity of skeletal muscles was recently recognized with the discovery of myostatin, a potent inhibitor of skeletal muscle cell proliferation and growth (10,11). Then, the term “myokines” was established and began to represent various other muscle-secreted factors as irisin (12). Also bone cells are able to secrete “osteokines” (FGF23 and sclerostin) into the bloodstream to affect distant targets (2,13). Muscles and bones have been considered as endocrine organs but their cross-talk with a clinical approach has been less investigated. Irisin has been considered one of the molecules responsible for muscle–bone connectivity and a potential target for sarcopenia and osteoporosis therapy (14).

Based on the developmental origins of health and disease theory, intrauterine and early-life events play a role for the risk of non-communicable chronic diseases (15). Insults occurring in the intrauterine environment can generate epigenetic changes that predispose to metabolic and cardiovascular disease later in life (16,17). There is some evidence on the association of birth weight and muscle strength and bone mass in adulthood (18-20), but other factors related to pre-pregnancy and pregnancy period were scarcely investigated. As far as we know, no study has examined early-life events with the musculoskeletal unit.

Muscle-bone relationships is a promising research area, and few clinical studies have explored interactions between these tissues in healthy young individuals

or deepened on clinical associated factors throughout life course. The Nutritionists' Health Study (NutriHS), conducted in young adults, offers an opportunity to: 1) evaluate whether parameters of muscle and bone compartments were associated; and 2) examine predictive factors of muscle and bone parameters related to events from the early life and current habits.

METHODS

- Study design, setting, and participants

Current study was performed using baseline data of the NutriHS, started in 2014. Recruitment and data collection occurred from 2015 to 2018. NutriHS is a cohort study that evaluates undergraduates and graduates from Nutrition courses in São Paulo State, Brazil. It was approved by School of Public Health ethics committee and, before to be included in study, all the participants signed the informed consent electronically, using the NutriHS website (www.fsp.usp.br/nutrihs). Previously, details were reported on its purposes and methodological aspects (21).

The present study has a mixed design. Once our early life events variables were recovered from the questionnaire, it was considered a historical cohort. Also, cross-sectional analyses on the associations with outcomes were done. Participants answered online questionnaires about sociodemographic data, family history, early life events, lifestyle, current diet and clinical data (22,23). The researcher team checked the information provided. A visit to the health care center of the School of Public Health of the University of São Paulo was scheduled. They were submitted to clinical examination and had biological samples collected. Muscle tests, bone densitometry and body composition were performed in a second visit.

A convenience sample of the first 201 participants of NutriHS who met eligibility criteria was taken. Inclusion criteria were: women aged 20 to 45 years, with regular menstruation, no past or current history of weight changes > 5% of total body weight in the last year, malignancy, elevated blood pressure, and disturbances of glucose and lipid metabolism. Exclusion criteria were pregnancy and anthropometric measures exceeding the limits of the densitometer (1.87 m and/or 201 kg, respectively). All participants answered the questionnaires and were submitted to anthropometry, bone densitometry and body composition by dual-energy x-ray

absorptiometry (DXA). Information about their birth weight was missing for 31 participants. A random sub-sample of 148 participants were submitted to blood pressure measurement and laboratory examination.

- Variables

Exposures were calf circumference (CC), appendicular skeletal muscle mass (ASM), appendicular skeletal muscle mass index (ASMI), handgrip, chair-stand test and gait speed. Outcome variables were bone mineral density and content (BMD and BMC) of the total body (TB), lumbar spine (LS), total femur (TF) and femoral neck (FN). Possible associated factors were maternal age in pregnancy, maternal pre-pregnancy BMI, maternal pregnancy weight gain, skin color, type of birth, birth weight, breastfeeding, menarche age, contraceptive use, corticosteroid use, calcium intake, smoking, alcohol use and physical activity.

Biochemical profile including 25-hydroxyvitamin D determination was also evaluated to characterize health status of our sample.

- *Clinical measurements*

Weight and height were measured, respectively, with digital scale (Filizola®, São Paulo, Brazil) to the nearst 0.1kg and with a fixed stadiometer with 0.1 cm precision, and then, BMI was calculated. CC was assessed with the participant in the orthostatic position at the mid-calf point. Blood pressure was taken in triplicate, using an automatic device (Omron model HEM-712C®, Omron Health Care Inc, USA), after resting in sitting position. Final values were those that represented the mean of the last two measurements.

- *Muscle mass, strength and performance*

Muscle strength was evaluated by the palmar grip strength, using a manual dynamometer (Jamar®), according to technique previously described (24). Measurements were made in duplicate in the dominant limb, respecting the interval of one minute to avoid muscular fatigue, and the mean value was considered. The participant was instructed to tighten the dynamometer with the highest possible force for three seconds and then release. The result was then read out.

Chair-stand test was obtained as a measure of legs' strength. Participant was asked to sit with arms crossed over the chest, and the time to get up and sit as fast

as possible five times was measured (25). In gait speed test, a muscle performance test, the participant was oriented to walk normally on a 4-meter course; time was checked, and results provided in meters per second (26).

- *DXA-derived measurements*

BMD and BMC of total body, lumbar spine (LS), femoral neck (FN) and total femur (TF) and lean mass of arms and legs were determined (GE Lunar® iDXA with EnCore sotware, Madison, WI, USA). Appendicular muscle mass (ASM) was obtained by the sum of the arms and legs lean masses. Appendicular muscle mass index (ASMI) was calculated dividing the ASM by squared height in meters. Bone densitometry and body composition scans were performed by the same researcher, certified by the Brazilian Association of Bone Evaluation and Osteometabolism (ABRASSO). Regions of interest (ROIs) were determined manually according to the manufacturer's specifications and calibrations were routinely made. Precision values and least significant change (LSC) were calculated according to the International Society for Clinical Densitometry (https://www.iscd.org/) after three successive measurements in fifteen women. The LSC of bone densitometry scans were: - LS BMD: 0.027 g/cm^2; - TF BMD: 0.017 g/cm^2; and - FN BMD: 0.056 g/cm^2. The precision values of body composition were: - TB BMD: 0.009 g/cm^2; - total lean mass: 275.0 g; and - ASM: 350.0 g.

- *Biochemical analyses*

Lipid profile (total cholesterol, LDL-cholesterol (LDL-c), HDL-cholesterol (HDL-c), triglycerides) was analyzed by colorimetric and enzymatic methods and LDL-c was calculated by the Friedwald equation. Fasting plasma glucose was obtained by the glucose oxidase method and insulin by immunofluorimetric assay, based on monoclonal antibody (Monobind, Inc., Lake Forest, CA, USA). Insulin resistance was estimated by the HOMA-IR index. 25-hydroxyvitamin were measured by electrochemiluminescence assay on Elecsys analyzers and Cobas modular platforms (Roche® Diagnostics, Indianapolis, USA).

- *Statistical analyses*

Data are expressed as mean and standard deviations (SD). The normality of the variables was verified by the Kolmogorov-Smirnov test. The correlation between variables was tested by Pearson coefficient. CC, ASMI and handgrip strength were

categorized in tertiles and variables of interest compared by ANOVA (post-hoc Bonferroni test). Associations of muscle compartment variables (exposure) with BMD and BMC (outcomes) were tested using multiple linear regression and Directed Acyclic Graph-recommended (DAG) minimal sufficient adjustments (27) (www.dagitty.net) (Figure 1). To analyze associations of factors of early and current life linear regression was also employed. Analyses were performed using STATA 13.1® statistical package. A *p* value < 0.05 was considered significant.

RESULTS

More than 70% of the sample reported white skin colour. Half had vaginal birth and the majority was breastfed for at least 6 months. Alcohol consumers and smokers were infrequent but most practiced physical activity (Table 1). About two thirds were using contraceptives. Their mothers' pre-pregnancy BMI and pregnancy weight gain were normal (Table 2). The sub-sample of 148 women had normal blood pressure levels and laboratory variables.

All bone variables were positively correlated to some muscle variables (CC, ASMI and handgrip strength). The strongest correlation coefficients were found for total body BMD and BMC (Figure 2).

Differences in the mean values of several bone sites were observed between the first and last tertile of CC, ASMI and handgrip strength (Table 3).

In linear regression models, direct associations between bone variables and CC, ASMI and HG strength were detected after adjustments for physical activity, skin color, BMI, menarche age and birth weight (Table 4).

Linear regression models for early- and current-life predictors of muscle and bone compartments showed the following associations:

a) birth weight with CC (β = 0.76, p= 0.003), ASMI (β = 0.17, p = 0.020), handgrip strength (β = 1.61, p < 0.001), lumbar spine BMD (β = 0.03, p = 0.032), total body BMD (β = 0.02, p = 0.043), total body BMC (β = 70.44, p = 0.003), total femur BMC (β = 0.88, p = 0.012);

b) physical activity with handgrip strength (β = 1.24, p = 0.009), total body BMD (β = 0.02, p = 0.048), total femur BMC (β = 0.83, p = 0.038);

c) smoking with CC (β = - 1.82, p= 0.029), ASMI (β = - 0.61, p = 0.023), total body BMD (β = - 0.05, p= 0.047), lumbar spine BMD (β = - 0.08, p = 0.038);

d) alcohol consumption with handgrip strength (β = - 1.57, p= 0.039).

Other lifetime factors (maternal age, pre-pregnancy BMI, pregnancy weight gain, skin color, type of birth, breastfeeding, menarche age, contraceptive and corticosteroid use, and calcium intake) showed no significant associations with muscle and bone parameters.

DISCUSSION

In a sample of Brazilian health young women, our findings reinforced direct associations between muscle and bone parameters, corroborating data from experimental and translational studies and suggesting that both tissues deserve concomitant assessments when aiming the prevention of osteosarcopenia. Also, associations detected with early-life factors and current habits highlighted the importance of assessing risk earlier in life.

Parallel increases of bone variables across tertiles of muscular compartment-related variables (CC, ASMI and handgrip strength) were interpreted as a muscle-bone synergism, which was coherent with the finding of strong associations detected in regression models. This cross-talk between tissues was previously demonstrated in animal studies in which mice paralyzed due to muscular dysgenesis in utero, presented alterations in the bone diaphysis (28). Other evidences came from studies in vitro through the discovery of several substances produced and secreted by muscles and bones (29, 30). However, in humans, this relationship muscle-bone is less explored (31, 32). In animals and humans, physical activity induced irisin production which has been suggested as one mechanism favoring metabolic benefits for both tissues (14).

We also use multiple linear regression searching for early- and current-life predictors of muscle and bone compartments. We found evidence that birth weight – as an indicator of the intrauterine environment quality – may play a role in musculoskeletal health. Despite limitations related to our study design, other prospective studies support this hypothesis (18-20). A contribution of our study was

to show that other early life factors (maternal age, pre-pregnancy BMI, pregnancy weight gain, skin color, type of birth, breastfeeding and menarche age) seems not useful to predict muscular or bone status in adulthood. Therefore, we speculate that intrauterine life might be the more critical and decisive for the osteomuscular development.

It is known the importance of vitamin D levels for bone and skeletal muscle metabolism (33). However, no correlation of bone and muscle variables with this vitamin was observed. Probably, this finding could be due to healthy status of our sample in general. Another finding that could be unexpected was the lack of association of bone variables and contraceptive use that was taken by 74% of our participants. Previous studies have indicated detrimental effect of this medication on the bone mass of adolescents and young women (34, 35). These contrasting results could be attributed to protective factors present in women from the NutriHS, who commonly practice physical activity and rarely smoke or consume alcohol. Sedentary lifestyle, smoking and alcohol consumption represent classic risk factors for major non-communicable chronic diseases including osteosarcopenia (36). Our sample is not representative of the subset of young women from developing countries where increasing rates of smoking and alcohol consumption were reported (37, 38). These behaviors have been fought to prevent cardiometabolic diseases and also osteometabolic ones.

The cross-sectional design and a relatively small sample size are limitations of our study. Despite the possibility of memory bias, self-reported data regarding early life events data have been commonly used in previous studies. Our selected sample impedes external validity of the study. However, data from a healthy and homogeneous sample (highly qualified white women) may be advantageous since could suggest the impact of a healthy life on muscles and bones status.

In conclusion, our findings in young adults support the muscle-bone crosstalk and suggest predictive factors (such as BW, physical activity, smoking and alcohol use) of muscle and bone compartments. This raises the importance of osteosarcopenia risk assessment early in life. Follow-up of the NutriHS participants could investigated such hypothesis.

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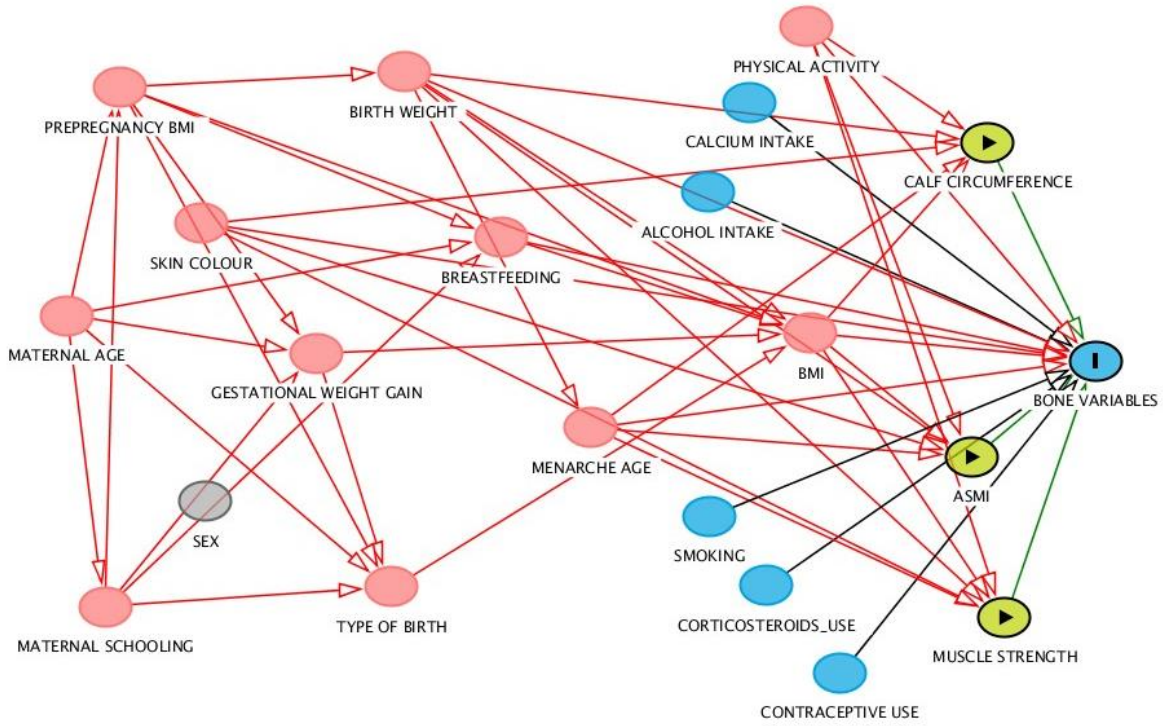

Figure 1. Directed Acyclic Graphs built for obtaining minimal sufficient adjustments for the total effect of muscle variables (calf circumference, ASMI and muscle strength) in bone variables. The minimal sufficient adjustment set included birth weight, BMI, menarche age, physical activity and skin colour.

Table 1. Maternal, neonatal period, and current characteristics of the participants of study.

Years of maternal education	0 to 4	23.9
	5 to 11	45.7
	≥ 12	30.4
Maternal smoking during pregnancy	yes	3.0
	no	97.0
Type of birth	vaginal	48.5
	cesarian section	51.5
Breastfeeding duration	< 6 months	28.6
	6 – 12 months	38.3
	≥ 12 months	33.1
Skin colour	white	72.4
	non-white	27.6
Current smoking	yes	8.2
	no	91.8
Current alcohol intake	yes	39.6
	no	60.4
Contraceptive use	yes	74.0
	no	26.0
Physical activity ≥ 150 min/week	yes	55.7
	no	44.3

Data expressed as number (%)

Table 2. Early-life, clinical and DXA data of participants of study.

	Entire sample n = 201	**Sub-sample n = 148**	**p-value**
Maternal pre-pregnancy BMI (kg/m^2)	22.5 (3.3)	22.1 (3.5)	0.757
Maternal pregnancy weight gain (kg)*	12.8 (9.0-15.0)	12.8 (9.0-15.0)	0.890
Birth weight (g)[#]	3,199 (424)	3,185 (422)	0.501
Age (years)*	23.0 (20.0-28.0)	22.0 (20.0-28.0)	0.847
Menarche age (years)*	12.1 (11.0-13.0)	12.0 (11.0-13.0)	0.962
Body mass index (kg/m^2)	22.9 (2.9)	23.0 (3.0)	0.417
Waist circumference (cm)	77.1 (8.3)	77.7 (8.6)	0.125
Fasting plasma glucose (mg/dL)	-	83.1 (9.3)	-
Total cholesterol (mg/dL)	-	172.2 (34.6)	-
Non HDL-cholesterol (mg/dL)	-	117.3 (32.0)	-
Triglycerides (mg/dL)	-	92.8 (47.8)	-
Insulin (μUI/L)	-	9.5 (4.2)	-
25-hydroxyvitamin D (ng/mL)	-	26.2 (11.1)	-
Calf circumference (cm)	35.8 (2.9)	-	-
ASM (kg)	16.9 (3.1)	-	-
ASMI (kg/m^2)	6.4 (0.9)	-	-
Muscle strength-handgrip (kg)	24.4 (5.0)	-	-
Chair-stand test (sec)	10.0 (2.1)	-	-
Gait speed (m/sec)	1.0 (0.1)	-	-
Total body BMD (g/cm^2)	1.1 (0.1)	-	-
Total body BMC (g)	2,306 (288)	-	-
Lumbar spine BMD (g/cm^2)	1.2 (0.1)	-	-
Lumbar spine BMC (g)	55.4 (16.6)	-	-
Total femur BMD (g/cm^2)	1.0 (0.1)	-	-
Total femur BMC (g)	29.8 (4.2)	-	-
Femoral neck BMD (g/cm^2)	1.0 (0.1)	-	-
Femoral neck BMC (g)	4.6 (0.7)	-	-

Data expressed as means and standard deviation or medians and interquartile range.
[#] Data available for 170 participants. * Mann-Whitney test was used.
ASM: appendicular muscle mass; ASMI: appendicular muscle mass index, BMD: bone mineral density; BMC bone mineral content.

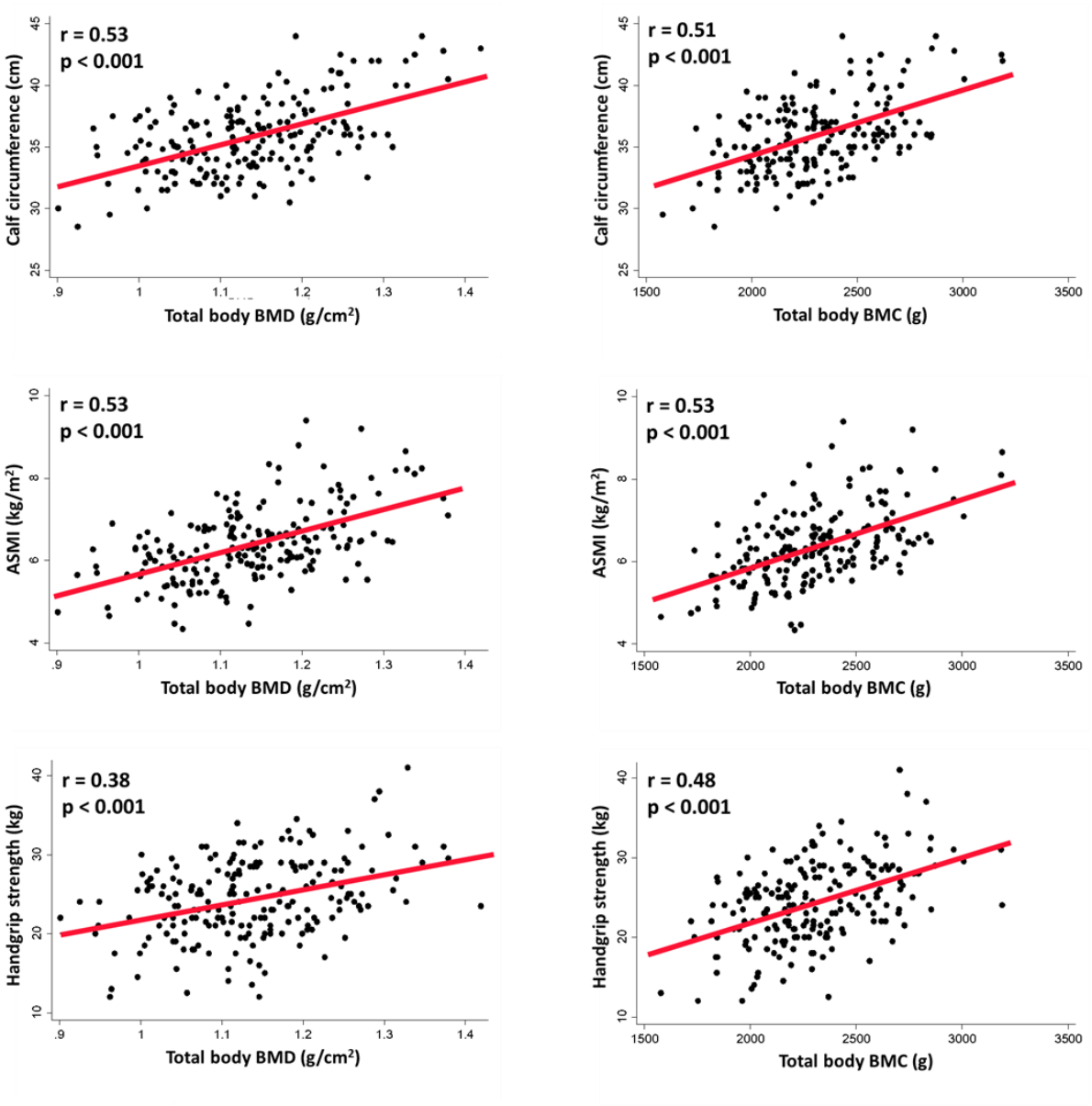

Figure 2. Correlations between muscle variables (calf circumference, ASMI and handgrip strength) and bone variables (total body BMD and BMC).

Pearson correlation coefficient used.
BMD: bone mineral density; BMC: bone mineral content; ASMI: appendicular muscle mass index.

Table 3. Mean values of bone variables for the entire sample (n = 201) according to muscle variables tertiles.

Calf circumference (cm)	**1st Tertile 29.5-34.4 n = 54**	**2nd Tertile 34.5-36.9 n = 62**	**3rd Tertile 37.0-49.5 n= 62**	**p-value**
LS BMD (g/cm²)	1.2 (0.2)	1.2 (0.1)	1.2 (0.1)	0.147
LS BMC (g)	56.3(12.1)	58.6 (15.1)	61.0 (14.5)	0.193
TB BMD (g/cm²)	1.1 (0.1)	1.1 (0.1)	1.2 (0.1)	<0.001
TB BMC (g)	2150.1(212.9)	2329.5(271.7)	2448.3(293.6)	<0.001
FN BMD (g/cm²)	1.0 (0.1)	1.0 (0.1)	1.0 (0.1)	0.019
FN BMC (g)	4.3 (0.7)	4.6 (0.6)	4.9 (0.7)	<0.001
TF BMD (g/cm²)	1.0 (0.1)	1.0 (0.1)	1.1 (0.1)	0.008
TF BMC (g)	27.9 (3.2)	29.7 (4.5)	31.9 (4.5)	<0.001
Appendicular muscle mass index (kg/m²)	**1st Tertile 4.33-6.01 n = 62**	**2nd Tertile 6.02-6.6 n = 63**	**3rd Tertile 6.7-9.7 n = 54**	**p-value**
LS BMD (g/cm²)	1.2 (0.2)	1.2 (0.1)	1.2 (0.1)	0.038
LS BMC (g)	54.8(13.7)	60.7(12.7)	59.6 (16.5)	0.053
TB BMD (g/cm²)	1.1 (0.1)	1.1 (0.1)	1.2 (0.1)	<0.001
TB BMC (g)	2149.8(236.2)	2324.6(247.7)	2492.4(279.5)	<0.001
FN BMD (g/cm²)	1.0 (0.1)	1.0 (0.1)	1.0 (0.1)	0.002
FN BMC (g)	4.3(0.7)	4.5 (0.5)	5.0 (0.7)	<0.001
TF BMD (g/cm²)	1.0 (0.1)	1.0 (0.1)	1.1 (0.1)	<0.001
TF BMC (g)	27.7 (3.3)	29.8 (3.6)	32.8 (4.2)	<0.001
Handgrip strength (kg)	**1st Tertile 12.0-21.9 n = 51**	**2nd Tertile 22.0-26.9 n = 73**	**3rd Tertile 27.0-41.0 n = 58**	**p-value**
LS BMD (g/cm²)	1.1 (0.1)	1.2 (0.1)	1.2 (0.2)	0.009
LS BMC (g)	56.0(12.8)	59.4(11.5)	59.2 (18.4)	0.365
TB BMD (g/cm²)	1.1 (0.1)	1.1 (0.1)	1.2 (0.1)	<0.001
TB BMC (g)	2176.4(229.0)	2283.5(281.5)	2478.4(266.6)	<0.001
FN BMD (g/cm²)	1.0 (0.1)	1.0 (0.1)	1.0 (0.1)	0.265
FN BMC (g)	4.4(0.6)	4.6 (0.8)	4.8 (0.6)	0.002
TF BMD (g/cm²)	1.0 (0.1)	1.0 (0.2)	1.1 (0.1)	0.093
TF BMC (g)	28.2 (3.4)	29.5 (4.2)	32.1 (4.1)	<0.001

BMD: bone mineral density; BMC: bone mineral content; TB: total body; LS: lumbar spine; TF: total femur and FN: femoral neck

Table 4. Linear regression models of muscle variables associated with bone variables regarding 201 participants.

	CRUDE								
	Calf circumference (cm)			Handgrip strength (kg)			ASMI (kg/m^2)		
	β	95%CI	p	β	95%CI	p	β	95%CI	p
TB BMD (g/cm^2)	0.017	0.013-0.021	<0.001	0.007	0.005-0.010	<0.001	0.056	0.043-0.068	<0.001
TB BMC (g)	50.190	38.288-62.092	<0.001	27.540	20.443-34.637	<0.001	171.16	132.55-209.77	<0.001
LS BMD (g/cm^2)	0.011	0.004-0.018	<0.001	0.008	0.004-0.012	0.014	0.046	0.024-0.068	<0.001
LS BMC (g)	1.010	0.227-1.794	0.012	0.330	-0.130-0.790	0.158	4.091	1.513-6.668	0.002
TF BMD (g/cm^2)	0.014	0.008-0.020	<0.001	0.004	0.001-0.008	0.034	0.052	0.033-0.071	<0.001
TF BMC (g)	0.654	0.479-0.830	<0.001	0.323	0.212-0.434	<0.001	2.579	2.017-3.140	<0.001
FN BMD (g/cm^2)	0.012	0.007-0.018	<0.001	0.004	0.000-0.007	<0.001	0.048	0.030-0.067	<0.001
FN BMC (g)	0.091	0.061-0.121	<0.001	0.044	0.025-0.062	<0.001	0.334	0.237-0.432	<0.001
	ADJUSTED								
	Calf circumference (cm)			Handgrip strength (kg)			ASMI (kg/m^2)		
	β	95%CI	p	β	95%CI	p	β	95%CI	P
TB BMD (g/cm^2)	0.011	0.003-0.018	0.006	0.006	0.003-0.009	<0.001	0.041	0.014-0.068	0.003
TB BMC (g)	49.411	26.313-72.509	<0.001	23.340	15.067-31.711	<0.001	204.83	124.13-285.53	<0.001
LS BMD (g/cm^2)	-0.001	-0.014-0.012	0.881	0.008	0.003-0.012	0.001	0.015	-0.032-0.062	0.531
LS BMC (g)	-0.158	-1.767-1.451	0.846	0.209	-0.388-0.807	0.489	-1.026	-6.788-4.735	0.725
TF BMD (g/cm^2)	0.011	-0.001-0.023	0.064	0.005	0.000-0.009	0.028	0.038	-0.005-0.080	0.080
TF BMC (g)	0.589	0.251-0.927	0.001	0.271	0.149-0.393	<0.001	2.865	1.700-4.031	<0.001
FN BMD (g/cm^2)	0.007	-0.004-0.019	0.222	0.003	-0.001-0.007	0.183	0.042	0.001-0.084	0.046
FN BMC (g)	0.085	0.024-0.146	0.006	0.041	0.018-0.063	<0.001	0.411	0.198-0.624	<0.001

BMD: bone mineral density; BMC: bone mineral content; TB: total body; LS: lumbar spine; TF: total femur and FN: femoral neck; ASMI: appendicular skeletal muscle mass index.

6. CONCLUSIONS

For the best of our knowledge, our first paper was pioneer in suggesting cutoff values for DXA-derived VAT mass and volume in a sample of Brazilian young healthy women. Using ROC curves, our findings reinforced that anthropometry is a valuable tool for the identification of increased VAT, since anthropometric measurements (BMI, WC and WHtR) or indexes (TyG-BMI and TyG-WC) corresponded to the best VAT AUCs. VAT cutoffs suggested to identify of abnormal risk factors – particularly anthropometric parameters and abnormal TyG derivates – were between the 50th (103 g for mass; 108 cm^3 for volume) and 75th percentiles (274 g for mass; 289 cm^3 for volume) of DXA-derived VAT. Therefore, based on mean values found in the present study, young women with VAT mass of 221 g and volume of 232 cm^3 or above should be aware for cardiometabolic risk, even with normal biochemical profile. The knowledge of such values of visceral adiposity is desirable for early assessment of cardiometabolic risk but should not be extrapolated to other populations. These thresholds seem able to disclose a mild visceral fat accumulation, prior the deterioration of glucose and lipid metabolism, reinforcing the role of VAT as an early cardiometabolic risk marker, although prospective data are needed to confirm the DXA-VAT ability to detect young at-risk individuals.

We also have innovated in our second paper because we are the first to investigate the association of the musculoskeletal unit functionality with BW – taken as a proxy of intrauterine environment – early in adulthood, without any manifestation of osteosarcopenia. The finding of independent associations with both, muscle (mass and strength), and bone (mineral density and content) parameters in young healthy women reinforce that the impact of BW might be early perceived. Additionally, our study was useful to provide DXA results of muscle and bone compartments, as well as parameters of strength and muscle performance, not previously described for a healthy stratus of the Brazilian women.

Our third paper support the muscle-bone crosstalk and suggest predictive factors (such as BW, physical activity, smoking and alcohol use) of muscle and bone compartments.

Our study has the limitations of the cross-sectional design that impede to infer causality and a relatively small sample size. The sample did not represent the female Brazilian young population considering that was composed of highly educated White women. However, data obtained in a high-quality equipment with precise software, from a healthy homogeneous sample, may be an important initial step to define reference values of DXA-determined VAT mass and volume (paper 1). Our selected sample impedes extrapolation of the results for Brazilian young female population. However, in the other hand, data from a healthy and homogeneous sample could represent also a strength of our study, since we describe an adequate musculoskeletal profile that may be useful for future comparisons. An important strength was DXA assessment of musculoskeletal unit functionality, i.e. muscle and bone parameters concomitantly (papers 2 and 3).

Our findings in young healthy women from the NutriHS are in line with the importance of the first 1,000 days of life for musculoskeletal health. A healthy intrauterine environment, as well as the development in infancy, should enable adequate muscle and bone mass peaks in adulthood reducing the risk of osteosarcopenia in elderly age. Therefore, we believe that success in musculoskeletal care depends on a multidisciplinary approach, focused on life course and intergerational epidemiologies, that is from conception to the end of life and in the next generations.

Further prospective studies, as NutriHS, are necessary to investigate the ability of BW to predict osteosarcopenia risk in the long-term, as well as to confirm the role of VAT as an early cardiometabolic risk marker.

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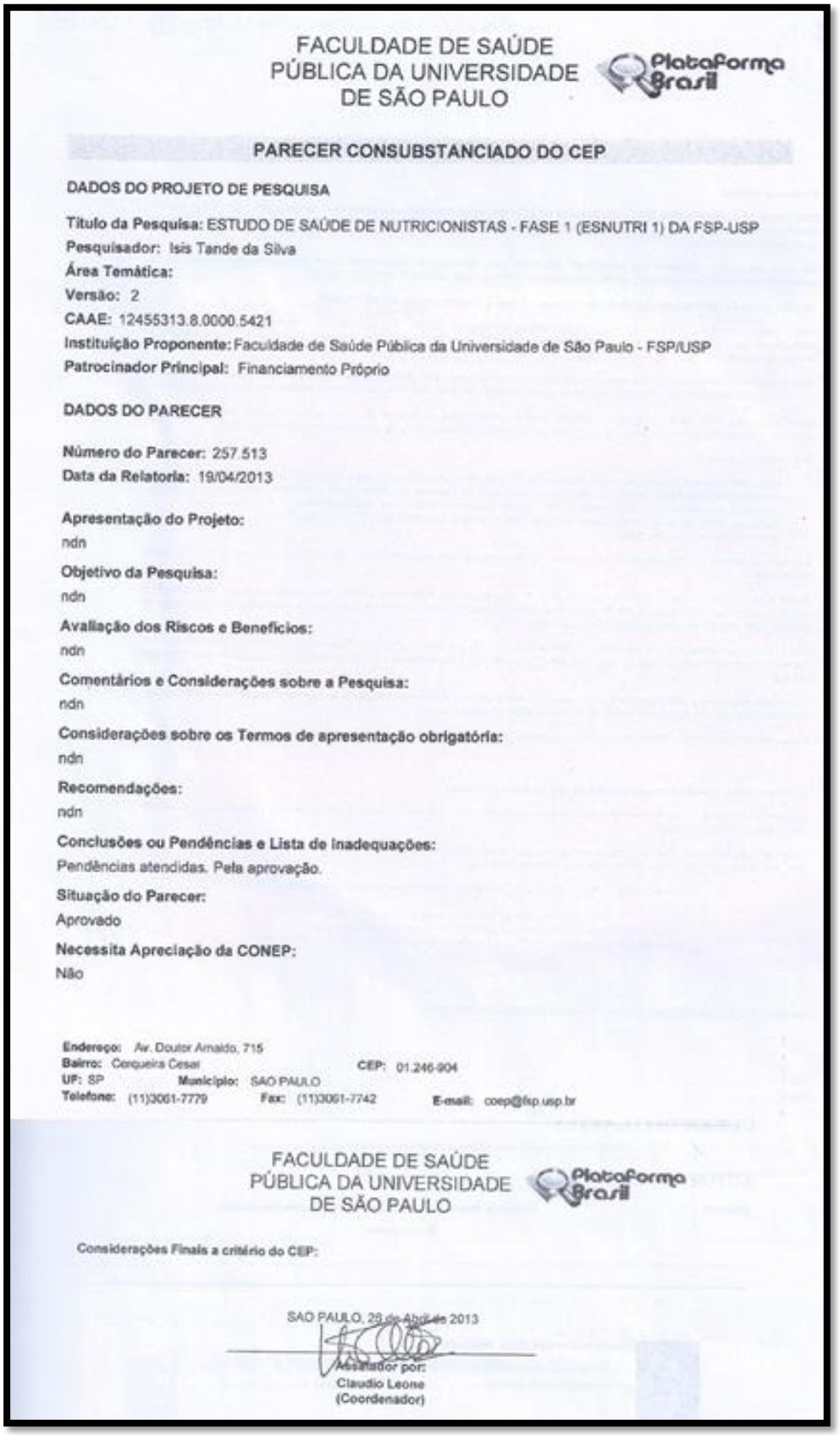
8. APPENDIX

Attachment 1: School of Public Health Ethics Committee



FACULDADE DE SAÚDE PÚBLICA DA UNIVERSIDADE DE SÃO PAULO

Plataforma Brasil

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: ESTUDO DE SAÚDE DE NUTRICIONISTAS - FASE 1 (ESNUTRI 1) DA FSP-USP
Pesquisador: Isis Tande da Silva
Área Temática:
Versão: 2
CAAE: 12455313.8.0000.5421
Instituição Proponente: Faculdade de Saúde Pública da Universidade de São Paulo - FSP/USP
Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 257.513
Data da Relatoria: 19/04/2013

Apresentação do Projeto:
ndn

Objetivo da Pesquisa:
ndn

Avaliação dos Riscos e Benefícios:
ndn

Comentários e Considerações sobre a Pesquisa:
ndn

Considerações sobre os Termos de apresentação obrigatória:
ndn

Recomendações:
ndn

Conclusões ou Pendências e Lista de Inadequações:
Pendências atendidas. Pela aprovação.

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

Endereço: Av. Doutor Arnaldo, 715
Bairro: Cerqueira Cesar **CEP:** 01.246-904
UF: SP **Município:** SAO PAULO
Telefone: (11)3061-7779 **Fax:** (11)3061-7742 **E-mail:** coep@fsp.usp.br

FACULDADE DE SAÚDE PÚBLICA DA UNIVERSIDADE DE SÃO PAULO

Plataforma Brasil

Considerações Finais a critério do CEP:

SAO PAULO, 29 de Abril de 2013

Assinador por:
Claudio Leone
(Coordenador)

<u>Attachment 2</u>: Informed Consent Form – Nutritionists' Health Study

Prezado participante,

Este termo de consentimento livre e esclarecido (TCLE) tem como finalidade esclarecê-lo a respeito dos objetivos, riscos e benefícios do presente estudo. Além disso, destaca que sua participação é livre e que sua privacidade será garantida em qualquer circunstância.

Ao clicar no botão "Consinto em participar" certifico que, após convenientemente esclarecido pelo pesquisador e ter entendido o que me foi explicado, aceito participar e concordo com os termos descritos no TCLE.

Título: Estudo de Saúde de Nutricionistas – Fase 1 (ESNutri 1) da FSP-USP

Este Termo descreve a finalidade, os procedimentos, benefícios, riscos, desconfortos e advertências deste estudo. É importante para sua decisão sobre a participação no estudo que leia e compreenda as explicações dos procedimentos propostos abaixo.

Objetivo: Avaliar a associação de variáveis de composição corporal com biomarcadores de risco cardiovascular; analisar a composição da microbiota intestinal e sua associação com hábitos alimentares e com biomarcadores de risco cardiovascular.

Benefício: Para indivíduos incluídos no estudo será a investigação de fatores de risco e/ou proteção nutricionais para doenças crônicas não-transmissíveis (DCNT), bem como fatores consagrados e os pouco explorados na literatura.

Sua participação inclui:

a) Responder questionário sobre saúde e ingestão alimentar via internet;

b) Realizar avaliação antropométrica, de pressão arterial e da composição corporal pelo densitômetro de dupla emissão com fonte de raio-X (DXA). Este exame é um método inócuo e preciso e sua realização leva 15 minutos.

c) Coleta de 30 ml de sangue com material descartável e pessoal treinado, após 12 horas de jejum;

d) Obtenção de amostra de fezes.

A coleta de sangue e o recebimento da amostra de fezes serão realizados no Centro de Saúde da FSP, em dia e hora pré-agendados.

Risco: Este estudo é considerado de risco mínimo. A antropometria, bem como o DXA, não causa qualquer desconforto e não requerem preparo prévio. A coleta de sangue pode raramente gerar um pequeno hematoma (manchas roxas) no local de punção, que, em geral, desaparecem após 3 a 5 dias.

Não haverá riscos para a integridade física, mental ou moral da sua pessoa. Todas as informações coletadas serão de caráter confidencial e utilizadas somente para fins científicos descritos no protocolo desta pesquisa, sem qualquer identificação pessoal.

O consentimento está sendo pedido exclusivamente para a participação neste estudo. É garantida e respeitada a privacidade na divulgação dos resultados da pesquisa, e não haverá sua identificação.

Liberdade: É garantido o direito de desistir a qualquer momento da participação nesta pesquisa, sem qualquer prejuízo. Não haverá qualquer tipo de relação ou influência de sua participação no projeto em sua vida acadêmica.

Informações para Contato em Caso de Intercorrências

Estaremos à disposição para informá-lo(a) sobre os procedimentos, riscos e benefícios decorrentes da pesquisa, ou qualquer outra dúvida sobre o estudo.

Caso haja quaisquer dúvidas ou perguntas relativas ao estudo, você poderá entrar em contato em qualquer momento com a Dra. Isis Tande da Silva e Dra. Sandra Roberta Gouvea Ferreira.

Telefones: 3061- 7705/7701 ou pelo e-mail: isistande@usp.br ou com o Comitê de Ética em Pesquisa – COEP/FSP

Telefones: 3061-7779 ou 3061-7742 e-mail: coep@fsp.usp.br

Endereço: Av. Dr. Arnaldo, no 715, Cerqueira César, São Paulo-SP, CEP:01246-904

<u>Attachment 3</u>: Socio-demographic, health and early life events questionnaires

1. <u>Avaliação Socioeconômica</u>

1.1 Nome:_____1.2 Universidade:_____

1.3 Cidade da Universidade:_____

1.4 Sexo: 1( ) M 2( ) F 1.5 Data de Nascimento:___/___/___

1.6 Endereço:_____No:_____ Apto:_____

Cidade/Estado:_____CEP:_____Telefone:_____e-mail_____

1.7 Mora com a família: 1( ) Sim 2( ) Não

1.8 Cor de pele: 1( ) Branca 2( ) Negra 3( ) Parda 4( ) Amarelo 5( ) indígena 6( ) Não sabe 7( ) Não quer informar

1.9 Qual período você está cursando ou qual o maior grau de formação que você atingiu?

1( ) 1º. Semestre 2( ) 2 º. Semestre 3( ) 3 º. Semestre 4( ) 4 º. Semestre 5 ( ) 5 º. Semestre 6 ( ) 6 º. Semestre 7 ( ) 7 º. Semestre 8 ( ) 8 º. Semestre 9 ( ) 9 º. Semestre 10 ( ) 10 º. Semestre 11 ( ) graduado 11 ( ) Especialista 12 ( ) Mestre 13 ( ) Doutor 15 ( ) Pós-doutorado

1.10 Estado civil: 1( ) solteiro(a) 2( )casado(a) ou união estável 3( )Divorciado 4( )Viúvo(a)

1.11 Reside com: 1( ) sozinha 2( )companheiro(a) ou esposo(a) 3( )pais ou responsáveis 4( ) amigos (república)

1.12 Qual a escolaridade atual de seu pai ou responsável?

1( ) Nunca frequentou escola 2( ) 1º. Grau incompleto (de 1 a 7 anos de estudo) 3( ) 1º. Grau completo (de 8 a 10 anos de estudo) 4( ) 2º. Grau completo (de 11 a 13 anos de estudo) 5( ) Universitário (14 ou mais anos de estudo 6( ) Pós-graduação

1.13 Renda familiar: 1( ) <1 SM – R$724,0 2( ) 1 - 5 SM 3( ) 6 - 10 SM 4( ) >10 SM 5( ) Não sabe

1.14 Quantas pessoas moram na sua casa? _____

1.15 Trabalha ou estagia: 1( ) Sim 2 ( ) Não.

Se sim, quantas horas em média por semana: _____

2. <u>Avaliação Clínica</u>

2.1 Usa medicamento: 1( ) Sim 2( ) Não. Se sim, qual: _____

2.2 Você apresenta alguma das doenças:

-Diabetes: 1( ) Sim 2( ) Não -Hipertensão: 1( ) Sim 2( ) Não

-Dislipidemia: 1( ) Sim 2( ) Não -Outra: 1( ) Sim 2( ) Não

Se sim, qual? _____

2.3 Existe algum familiar de primeiro grau (pai, mãe, irmão(s) e irmã(s)) com:

Diabetes 1( ) Sim 2( ) Não

Se sim, Quem?_____

Hipertensão 1( ) Sim 2( ) Não

Se sim, Quem?_____

Obesidade 1( ) Sim 2( ) Não

Se sim, Quem?_____

AVC 1( ) Sim 2( ) Não

Se sim, Quem?_____

2.5 Segue alguma dieta específica 1( ) Sim 2( ) Não

Se sim, Qual?_____

2.8 Qual seu consumo de bebidas alcoólicas

1( )1 a 2 dias por semana 2( ) 3 a 4 dias por semana 3( ) 5 a 6 dias por semana 4( ) todos os dias inclusive sábado e domingo 5( ) menos de 1 dia por mês 6( ) menos de 1 dia por semana

2.9 Fumo: 1( ) Não 2( ) Sim, diariamente 3( ) Sim, mas não diariamente 4( ) No passado

2.10 Peso: _____Kg

2.12 Altura: _____m

3. <u>Questionário de fatos precoces da vida</u>

As questões abaixo são relacionadas a fatos precoces da sua vida. Poderá ser necessário CONSULTAR SUA MÃE OU RESPONSÁVEL para obter as informações.

Esse questionário ficará disponível para que complete assim que tenha o conhecimento dos dados solicitados, para aqueles que não são possíveis de se obter você deve assinalar “não sei”.

3.1 Qual a idade de sua mãe na época que você nasceu? ____anos. ( ) Não sei

3.2 Qual a estatura (altura) de sua mãe? _____m. ( ) Não sei

3.3 Qual a escolaridade de sua mãe (ou responsável) na época que você nasceu? ( ) Nunca frequentou escola ( ) 1º. Grau incompleto (de 1 a 7 anos de estudo) ( ) 1º. Grau completo (de 8 a 10 anos de estudo) ( ) 2º. Grau completo (de 11 a 13 anos de estudo) ( ) Universitário (14 ou mais anos de estudo ( ) Pós-graduação

3.4 Antes da gestação sua mãe fazia tratamento medicamentoso para: ( ) Não ( ) Asma ( )Doença Auto-imune ( )Diabetes Tipo1 ( )Não sei ( )Outro Qual: _____

3.5 A sua gestação foi a primeira de sua mãe? ( ) Sim ( ) Não, foi a segunda ( ) Não, foi a terceira ou mais ( ) Não sei

3.6 Qual o peso (aproximado) de sua mãe quando ficou grávida de você?____Kg. ( ) Não sei

3.7 Quantos kg sua mãe engordou na sua gestação?_____Kg. ( ) Não sei

3.8 Houve algum problema de saúde com sua mãe durante a gestação?

( ) Não ( ) Pressão Alta ( ) Diabetes Gestacional ( ) Eclampsia ( ) Tabagismo ( ) Não sei ( ) Outro Qual: _____

3.9 Você nasceu por qual tipo de parto? ( ) Natural ( ) Cesária ( ) Não sei

3.10 Você foi um bebê prematuro, isso é, nasceu antes do previsto?

( ) Não ( ) Sim ( ) Não sei

3.11 Qual foi seu peso ao nascer?_____ Kg. ( ) Não sei

3.12 Você tem irmão(s) gêmeo(s)? ( ) Não ( ) Sim ( ) Não sei

3.13 Você recebeu aleitamento materno? ( ) Não ( ) Não sei

( )Sim, por quantos meses: _____

3.13 Com quantos meses foi introduzido fórmula, leite de vaca ou outro tipo de alimento?_____

3.14 Durante a infância e adolescência você foi considerado (a) acima do peso ideal em uma ou mais das faixas etárias abaixo? ( ) Não, nunca fui considerado(a) com excesso de peso ( ) Sim ( )Não sei

Se sim, quando (você pode assinalar mais do que 1 item): ( ) 0 a 2 anos de idade ( ) 3 a 6 anos de idade ( ) 7 a 12 anos de idade ( ) acima de 12 anos de idade

3.15 Quando criança você fazia tratamento medicamentoso para: ( ) Não fazia nenhum tipo de tratamento contínuo ( ) Asma ( ) Doença Auto-imune ( )Diabetes Tipo1 ( )Não sei ( ) Outro. Qual:_____

3.16 Quando criança você tinha o hábito de comer frutas e verduras? ( ) Não ( ) Sim ( ) Não sei

3.17 Quando criança você praticava atividade física programada (além da grade curricular da escola) pelo menos 2 vezes por semana? ( ) Não ( ) Não sei ( ) Sim, qual(is):_____

Attachment 4: Confirmation e-mail and body composition and bone densitometry appointment

Olá!

Gostaríamos de agradecer por sua participação no NutriHS e de relembrar que seus exames de COMPOSIÇÂO CORPORAL e DENSITOMETRIA estão agendados para o dia __/__/__ , _____-FEIRA às ___:___h.

POR FAVOR, CHEGAR COM 15 MINUTOS DE ANTECEDÊNCIA.

Seguem abaixo as **orientações e restrições** para realizar os exames de Composição Corporal e Densitometria:

- Usar roupas **SEM** metal. Não poderá haver metal nem mesmo nas roupas íntimas. Para as mulheres, seria ideal o uso de top de ginástica. Caso prefira, você poderá trocar de roupa no local do exame.

- **Não** poderão realizar o exame **mulheres grávidas** ou **com suspeita de gravidez**.

- **Não** ter realizado **exames contrastados** nas **últimas 2 semanas** antes do exame de composição corporal.

- Medicamentos à base de cálcio, deverão ser **suspensos 24 horas** antes da realização do exame.

- **Não** realizar uma **grande refeição ou ingerir muito líquido** pelo menos 2 horas antes do exame.

- Esvaziar a bexiga antes da realização do exame.

Local do exame:

Faculdade de Saúde Pública da USP

Prédio da Biblioteca – segundo andar / sala 5

Av. Dr. Arnaldo, 715 - São Paulo - Metrô Clínicas

Por favor, caso não possa comparecer nesta data, acesse nosso site e faça seu reagendamento!

Atenciosamente,

Equipe NutriH

A/C Profª Tit. Sandra R. G. F. Vivolo
Departamento de Nutrição
Av. Dr. Arnaldo, 715 - Cerqueira Cesar
São Paulo - SP - Brasil - CEP - 01246-904

Attachment 5: Body composition assessment

Estudo de Saúde de Nutricionistas
"Conhecendo nutrição, gerando saúde"

Associations of birth weight with DXA-determined body composition, bone densitometry and cardiometabolic risk profile in young women from the Nutritionists' Health

Data da Avaliação: __/__/__

Número NutriHS : _____Data de Nascimento : ___/___/__ Sexo : ____ Faculdade_____

Nome :

_____

e-mail : _____ Fones :

_____

Checagem de contra-indicações para realização da DXA:

Gestação atual? _____ Suspeita de gestação? _____ DUM : ____/____/____

Exame contrastado ou de medicina nuclear nos últimos 2 semanas?

_____

Prótese metálica? _____Prótese de silicone : □ Não □ Sim : □ Mamas □ Glúteos

Checagem do Questionário de Eventos Precoces:

Antecedentes Pessoais:

ATIVIDADE FÍSICA	TIPO	FREQUÊNCIA	TEMPO
Atual			
Infância / adolescência			

	□ TABAGISMO ATUAL	□ TABAGISMO PRÉVIO
Há / Por quanto tempo?		
Cigarros / dia :		

	QUANTIDADE	FREQUÊNCIA	TIPO
Ingestão alcoólica			

MEDICAMENTOS	ATUALMENTE	PREVIAMENTE
ANTICONCEPCIONAL		
CORTICÓIDES		
SUPLEMENTO DE CÁLCIO		
SUPLEMENTO DE PROTEÍNA		

ANABOLIZANTE		
POLIVITAMÍNICOS		
OUTROS		

OBESIDADE NA INFÂNCIA/ ADOLESCÊNCIA	**IDADE**
□ SIM □ NÃO	□ 0 a 2 anos □ 3 a 6 anos □ 7 a 12 anos □ <12 anos

IDADE DA MENARCA	**CICLOS MENSTRUAIS REGULARES**	**PERÍODOS DE AMENORRÉIA**

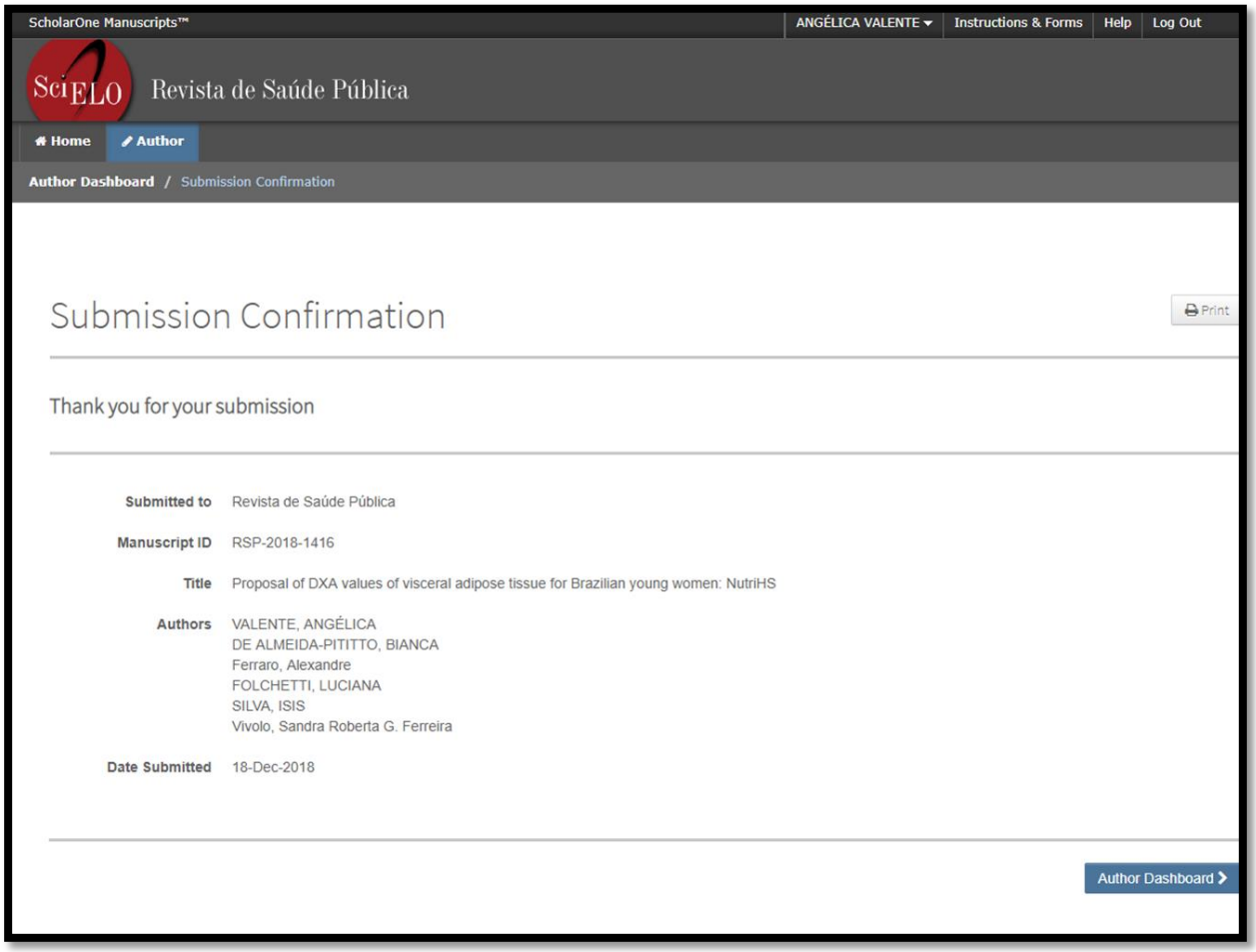
PATOLOGIA	**ANTECEDENTES PESSOAIS**	**ANTECEDENTES FAMILIARES**
OSTEOPOROSE		
FRATURAS		
DOENÇA CARDIOVASCULAR		
DIABETES MELITUS		
DISLIPIDEMIA		
HAS		
HIPOTIREOIDISMO		
HIPERTIREOIDISMO		
CIRURGIAS		
HOSPITALIZAÇÃO		
OUTROS		

<u>AVALIAÇÃO FÍSICA</u>:

Peso (Kg)	Estatura (m)
Circunferência abdominal (cm)	Circunferência abdominal (cm)
Circunferência da panturrilha (cm)	Obs.:

FORÇA DE PREENSÃO PALMAR	**Primeira aferição** (kg)	**Segunda aferição** (kg)
Membro dominante □ Direito □ Esquerdo		
TESTE DE LEVANTAR-SE DA CADEIRA	Tempo (segundos)	
GAIT SPEED TEST	Tempo (segundos)	

Attachment 6: Submitted to Journal of PublicHealth



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SciELO Revista de Saúde Pública

Home | Author

Author Dashboard / Submission Confirmation

Submission Confirmation

Print

Thank you for your submission

Submitted to	Revista de Saúde Pública
Manuscript ID	RSP-2018-1416
Title	Proposal of DXA values of visceral adipose tissue for Brazilian young women: NutriHS
Authors	VALENTE, ANGÉLICA DE ALMEIDA-PITITTO, BIANCA Ferraro, Alexandre FOLCHETTI, LUCIANA SILVA, ISIS Vivolo, Sandra Roberta G. Ferreira
Date Submitted	18-Dec-2018

Author Dashboard

Attachment 7: Submitted to Journal of Clinical Densitometry

Dear Dr Marques Martins Valente,

Submission no: JCLINDENSITOM_2018_177
Submission title: Birth weight is associated with DXA-determined muscle-bone unit in young healthy women from the Nutritionists' Health Study
Corresponding author: Professor Sandra Ferreira
Listed co-author(s): Dr Angélica Marques Martins Valente, Dr. Bianca Almeida-Pititto, Professor Alexandre Archanjo Ferraro, Dr Isis Tande Silva, Dr Luciana G D Folchetti

Professor Ferreira has submitted a manuscript to Journal of Clinical Densitometry and listed you as a co-author. This email is to let you know we will be in contact with updates at each decision stage of the submission process.

The link below takes you to a webpage where you can sign in to our submission system using your existing Elsevier profile credentials or register to create a new profile. You will then have the opportunity to tailor these updates and view reviewer and editor comments once they become available.

http://www.evise.com/profile/api/navigate/JCLINDENSITOM?resourceUrl=%2Fco-author%2F%3Fdgcid%3Dinvite_email_coauthoroutreach10946950%23%2FJCLINDENSITOM%2Fsubmission%2FJCLINDENSITOM_2018_177

If you are not a co-author of this manuscript, please contact Researcher Support at: https://service.elsevier.com

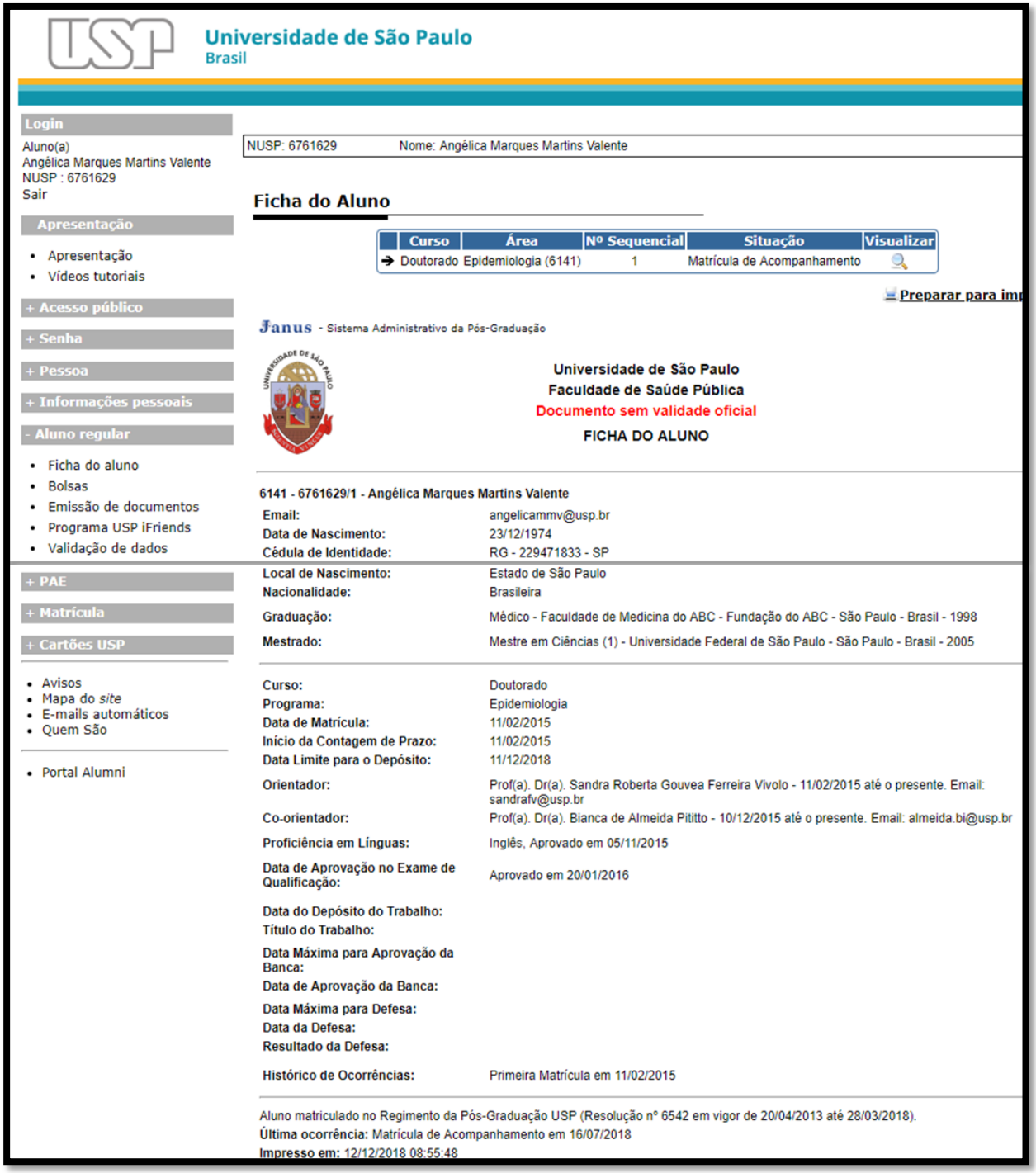
Thank you very much for your submission and we will be in touch as soon as we have any news to share.

Journal of Clinical Densitometry

If you do not wish to receive further update emails on your co-authored submission, you can unsubscribe via this link:

http://www.evise.com/co-author/#/JCLINDENSITOM/unsubscribe/angelicammvalente@uol.com.br/0TvjBRktcElxwGBUJsDthpIU-BiZYmuNAZ_JMN2e93IPI1TCeknqOvMubJ220-kGMfyeVh3PuyxSrNsdRSYgddqAVizZj53IWtgSl0jcgCI

<u>Attachment 8</u>: University of São Paulo records of the PhD candidate (Janus System)



Universidade de São Paulo
Brasil

Login
Aluno(a)
Angélica Marques Martins Valente
NUSP : 6761629
Sair

Apresentação
- Apresentação
- Vídeos tutoriais

+ Acesso público
+ Senha
+ Pessoa
+ Informações pessoais
- Aluno regular
- Ficha do aluno
- Bolsas
- Emissão de documentos
- Programa USP iFriends
- Validação de dados

+ PAE
+ Matrícula
+ Cartões USP

- Avisos
- Mapa do *site*
- E-mails automáticos
- Quem São

- Portal Alumni

NUSP: 6761629 Nome: Angélica Marques Martins Valente

Ficha do Aluno

Curso	Área	Nº Sequencial	Situação	Visualizar
➔ Doutorado	Epidemiologia (6141)	1	Matrícula de Acompanhamento	

Preparar para im

Janus - Sistema Administrativo da Pós-Graduação

Universidade de São Paulo
Faculdade de Saúde Pública
Documento sem validade oficial
FICHA DO ALUNO

6141 - 6761629/1 - Angélica Marques Martins Valente

Email:	angelicammv@usp.br
Data de Nascimento:	23/12/1974
Cédula de Identidade:	RG - 229471833 - SP
Local de Nascimento:	Estado de São Paulo
Nacionalidade:	Brasileira
Graduação:	Médico - Faculdade de Medicina do ABC - Fundação do ABC - São Paulo - Brasil - 1998
Mestrado:	Mestre em Ciências (1) - Universidade Federal de São Paulo - São Paulo - Brasil - 2005
Curso:	Doutorado
Programa:	Epidemiologia
Data de Matrícula:	11/02/2015
Início da Contagem de Prazo:	11/02/2015
Data Limite para o Depósito:	11/12/2018
Orientador:	Prof(a). Dr(a). Sandra Roberta Gouvea Ferreira Vivolo - 11/02/2015 até o presente. Email: sandrafv@usp.br
Co-orientador:	Prof(a). Dr(a). Bianca de Almeida Pititto - 10/12/2015 até o presente. Email: almeida.bi@usp.br
Proficiência em Línguas:	Inglês, Aprovado em 05/11/2015
Data de Aprovação no Exame de Qualificação:	Aprovado em 20/01/2016
Data do Depósito do Trabalho:	
Título do Trabalho:	
Data Máxima para Aprovação da Banca:	
Data de Aprovação da Banca:	
Data Máxima para Defesa:	
Data da Defesa:	
Resultado da Defesa:	
Histórico de Ocorrências:	Primeira Matrícula em 11/02/2015

Aluno matriculado no Regimento da Pós-Graduação USP (Resolução nº 6542 em vigor de 20/04/2013 até 28/03/2018).
Última ocorrência: Matrícula de Acompanhamento em 16/07/2018
Impresso em: 12/12/2018 08:55:48

Janus - Sistema Administrativo da Pós-Graduação

Universidade de São Paulo
Faculdade de Saúde Pública
Documento sem validade oficial
FICHA DO ALUNO

6141 - 6761629/1 - Angélica Marques Martins Valente

Sigla	Nome da Disciplina	Início	Término	Carga Horária	Cred.	Freq.	Conc.	Exc.	Situação
HEP5800-3/9	Bioestatística	02/03/2015	13/05/2015	90	6	100	B	N	Concluída
HEP5796-2/1	Princípios da Epidemiologia	03/03/2015	14/05/2015	90	6	100	A	N	Concluída
HEP5795-3/1	Delineamento e Introdução a Análise Epidemiológica	19/05/2015	29/06/2015	60	4	91	A	N	Concluída
MPE5748-2/3	Epidemiologia do Ciclo Vital: A Influência das Primeiras Décadas de Vida Sobre as Patologias do Adulto (Faculdade de Medicina - Universidade de São Paulo)	11/06/2015	01/07/2015	45	3	100	A	N	Concluída
MCG5825-4/5	Pedagogia Médica e Didática Especial (Faculdade de Medicina - Universidade de São Paulo)	01/03/2016	02/05/2016	90	6	90	A	N	Concluída
PSP5125-1/1	Estatística não Paramétrica	17/05/2016	27/06/2016	60	4	86	B	N	Concluída
PSP5103-1/1	Modelos de Regressão Aplicados em Epidemiologia I	02/08/2016	05/09/2016	60	4	83	A	N	Concluída
PSP5104-1/1	Modelos de Regressão Aplicados em Epidemiologia II	13/09/2016	24/10/2016	60	4	80	B	N	Concluída
PSP5105-1/1	Modelos de Regressão Aplicados em Epidemiologia III	01/11/2016	05/12/2016	60	4	80	A	N	Concluída
EPI5713-1/1	Introdução ao R para a Análise de Dados	22/05/2017	03/07/2017	30	2	88	A	N	Concluída
PSP5123-1/2	Análise Multinível em Estudos Epidemiológicos	15/08/2017	26/09/2017	60	0	-	-	N	Matrícula cancelada
HNT5758-3/1	Síndrome Metabólica: Fisiopatologia, Epidemiologia e Controle	03/04/2018	08/05/2018	30	0	-	-	N	Matrícula cancelada

	Créditos mínimos exigidos		Créditos obtidos
	Para exame de qualificação	Para depósito de tese	
Disciplinas:	0	16	43
Estágios:			
Total:	0	16	43

Créditos Atribuídos à Tese: 152

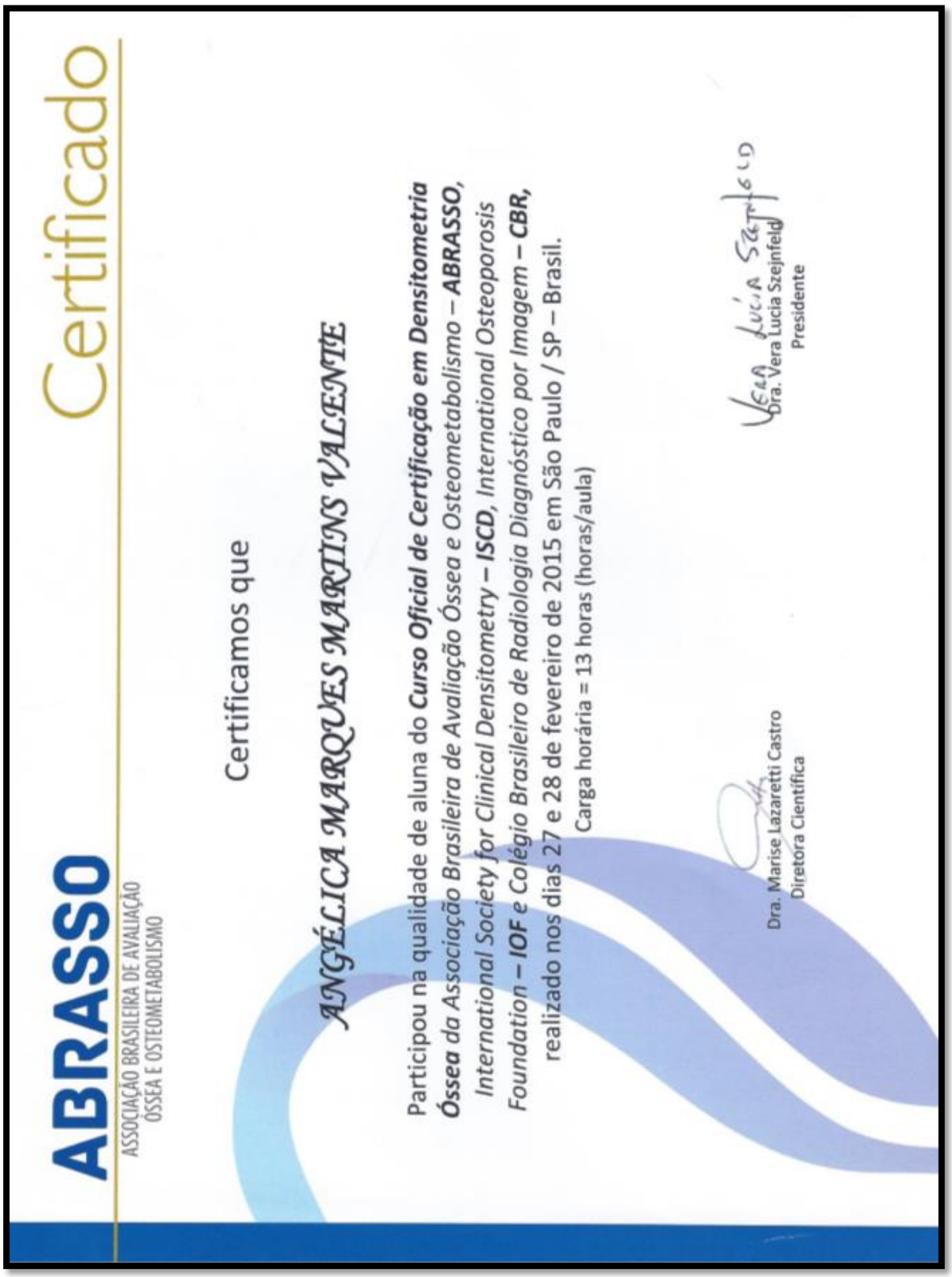
Observações:
1) Curso com validade nacional, de acordo com o disposto na Portaria MEC nº 1.077, de 31.08.2012..

Conceito a partir de 02/01/1997:
A - Excelente, com direito a crédito; B - Bom, com direito a crédito; C - Regular, com direito a crédito; R - Reprovado; T - Transferência.
Um(1) crédito equivale a 15 horas de atividade programada.

Última ocorrência: Matrícula de Acompanhamento em 16/07/2018
Impresso em: 12/12/2018 08:55:48

<u>Attachment 9</u>: Certificates

<u>Attachment 9.1</u>: Certificate "Curso Oficial de Certificação em Densitometria Óssea – ABRASSO", 2015.



ABRASSO
ASSOCIAÇÃO BRASILEIRA DE AVALIAÇÃO ÓSSEA E OSTEOMETABOLISMO

Certificado

Certificamos que

ANGÉLICA MARQUES MARTINS VALENTE

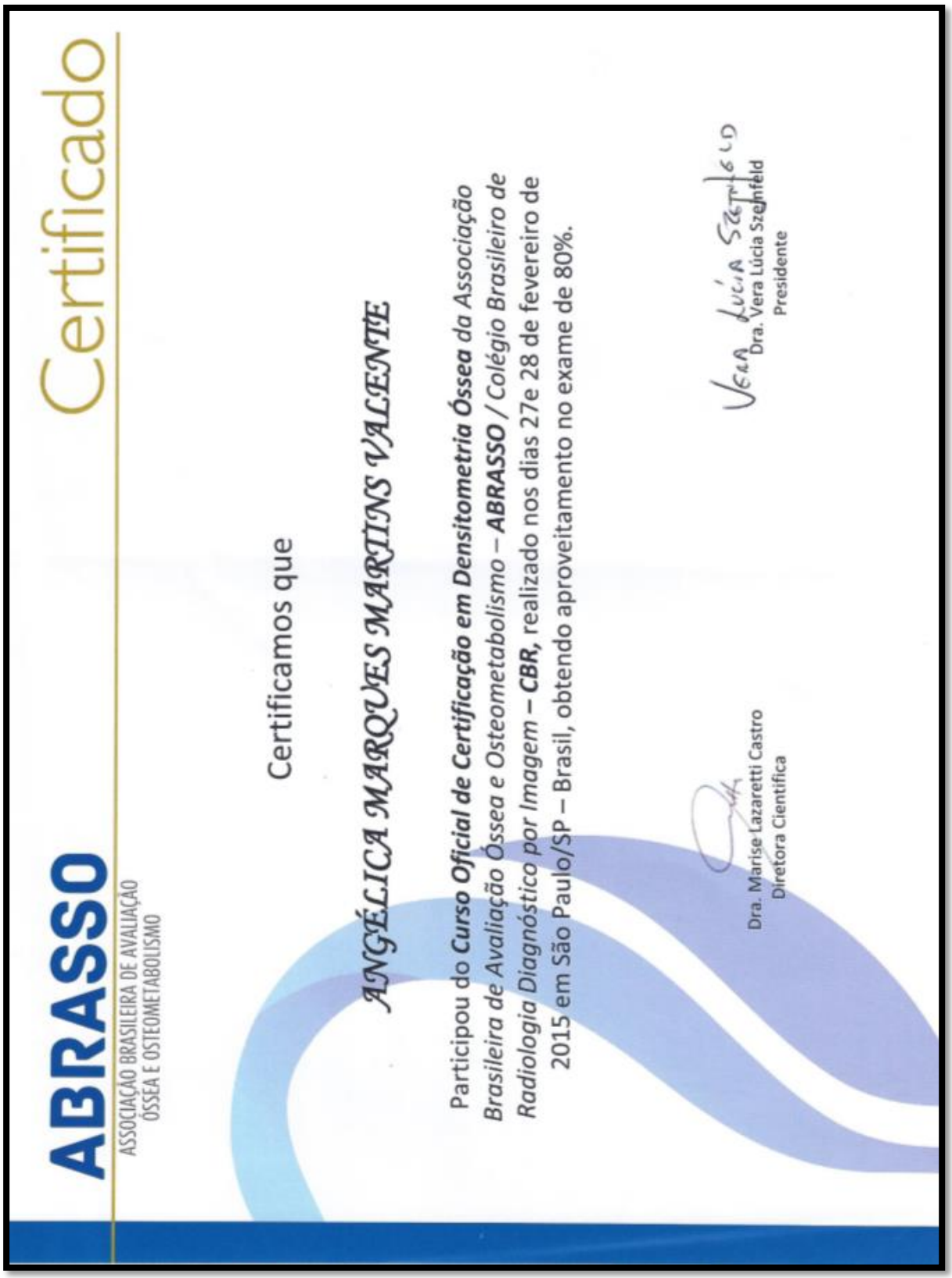
Participou na qualidade de aluna do ***Curso Oficial de Certificação em Densitometria Óssea*** *da Associação Brasileira de Avaliação Óssea e Osteometabolismo –* ***ABRASSO,*** *International Society for Clinical Densitometry –* ***ISCD,*** *International Osteoporosis Foundation –* ***IOF*** *e Colégio Brasileiro de Radiologia Diagnóstico por Imagem –* ***CBR,*** realizado nos dias 27 e 28 de fevereiro de 2015 em São Paulo / SP – Brasil.

Carga horária = 13 horas (horas/aula)

Dra. Marise Lazaretti Castro
Diretora Científica

Dra. Vera Lucia Szejnfeld
Presidente

<u>Attachment 9.2</u>: Certificate "Prova Oficial de Certificação em Densitometria Óssea – ABRASSO", 2015.



ABRASSO

ASSOCIAÇÃO BRASILEIRA DE AVALIAÇÃO ÓSSEA E OSTEOMETABOLISMO

Certificado

Certificamos que

ANGÉLICA MARQUES MARTINS VALENTE

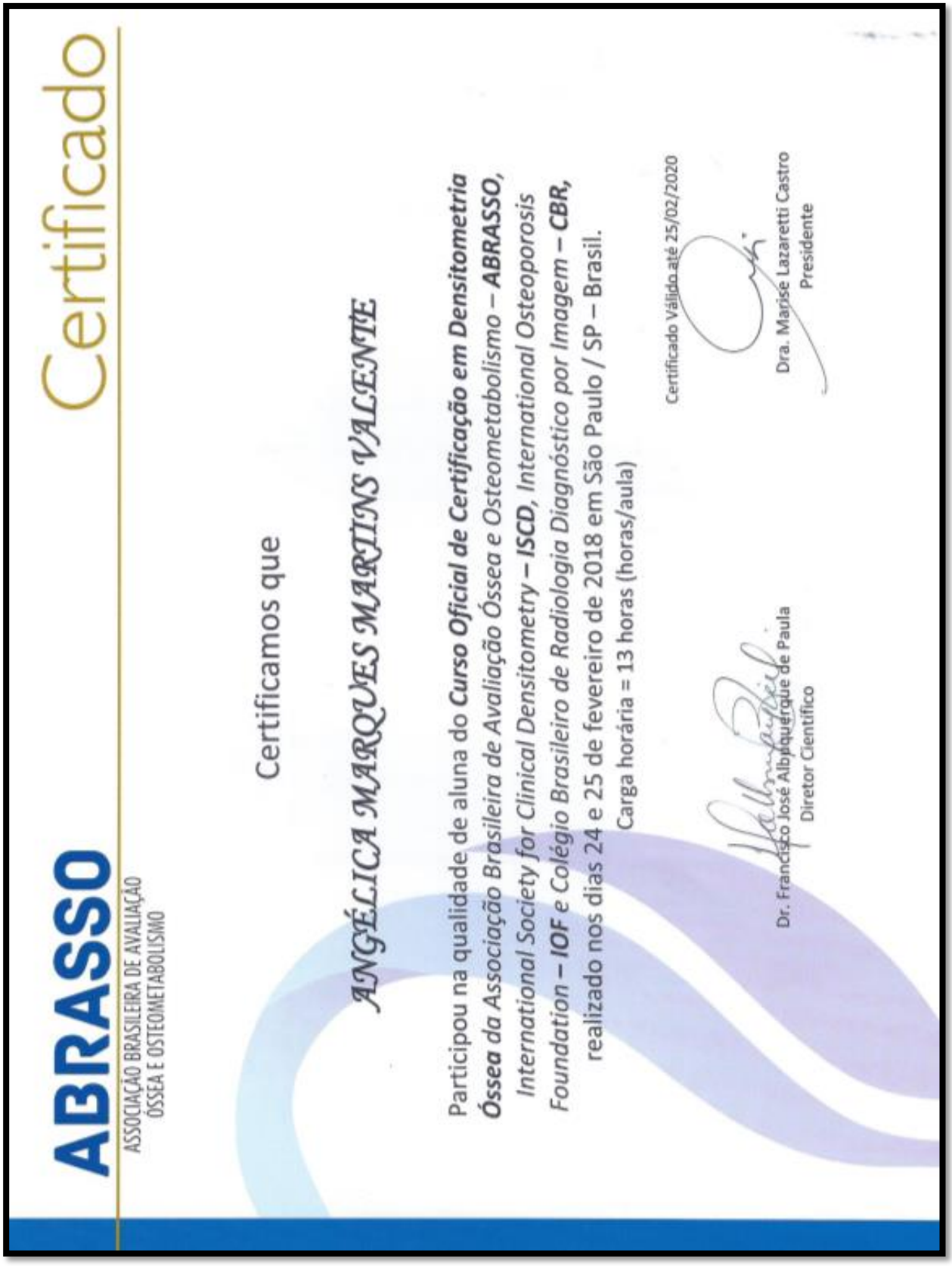
Participou do ***Curso Oficial de Certificação em Densitometria Óssea*** *da Associação Brasileira de Avaliação Óssea e Osteometabolismo –* ***ABRASSO*** */ Colégio Brasileiro de Radiologia Diagnóstico por Imagem –* ***CBR,*** realizado nos dias 27e 28 de fevereiro de 2015 em São Paulo/SP – Brasil, obtendo aproveitamento no exame de 80%.

Vera Lúcia Szejnfeld

Dra. Vera Lúcia Szejnfeld
Presidente

Dra. Marise Lazaretti Castro
Diretora Científica

<u>Attachment 9.3</u>: Certificate "Curso Oficial de Certificação em Densitometria Óssea – ABRASSO", 2018.



ABRASSO

ASSOCIAÇÃO BRASILEIRA DE AVALIAÇÃO ÓSSEA E OSTEOMETABOLISMO

Certificado

Certificamos que

ANGÉLICA MARQUES MARTINS VALENTE

Participou na qualidade de aluna do ***Curso Oficial de Certificação em Densitometria Óssea*** *da Associação Brasileira de Avaliação Óssea e Osteometabolismo –* ***ABRASSO,*** *International Society for Clinical Densitometry –* ***ISCD,*** *International Osteoporosis Foundation –* ***IOF*** *e Colégio Brasileiro de Radiologia Diagnóstico por Imagem –* ***CBR,*** realizado nos dias 24 e 25 de fevereiro de 2018 em São Paulo / SP – Brasil.

Carga horária = 13 horas (horas/aula)

Certificado Válido até 25/02/2020

Dr. Francisco José Albuquerque de Paula
Diretor Científico

Dra. Marise Lazaretti Castro
Presidente

<u>Attachment 9.4</u>: Certificate "Prova Oficial de Certificação em Densitometria Óssea – ABRASSO", 2018.

ABRASSO

ASSOCIAÇÃO BRASILEIRA DE AVALIAÇÃO ÓSSEA E OSTEOMETABOLISMO

Certificado

Certificamos que

ANGELICA MARQUES MARTINS VALENTE

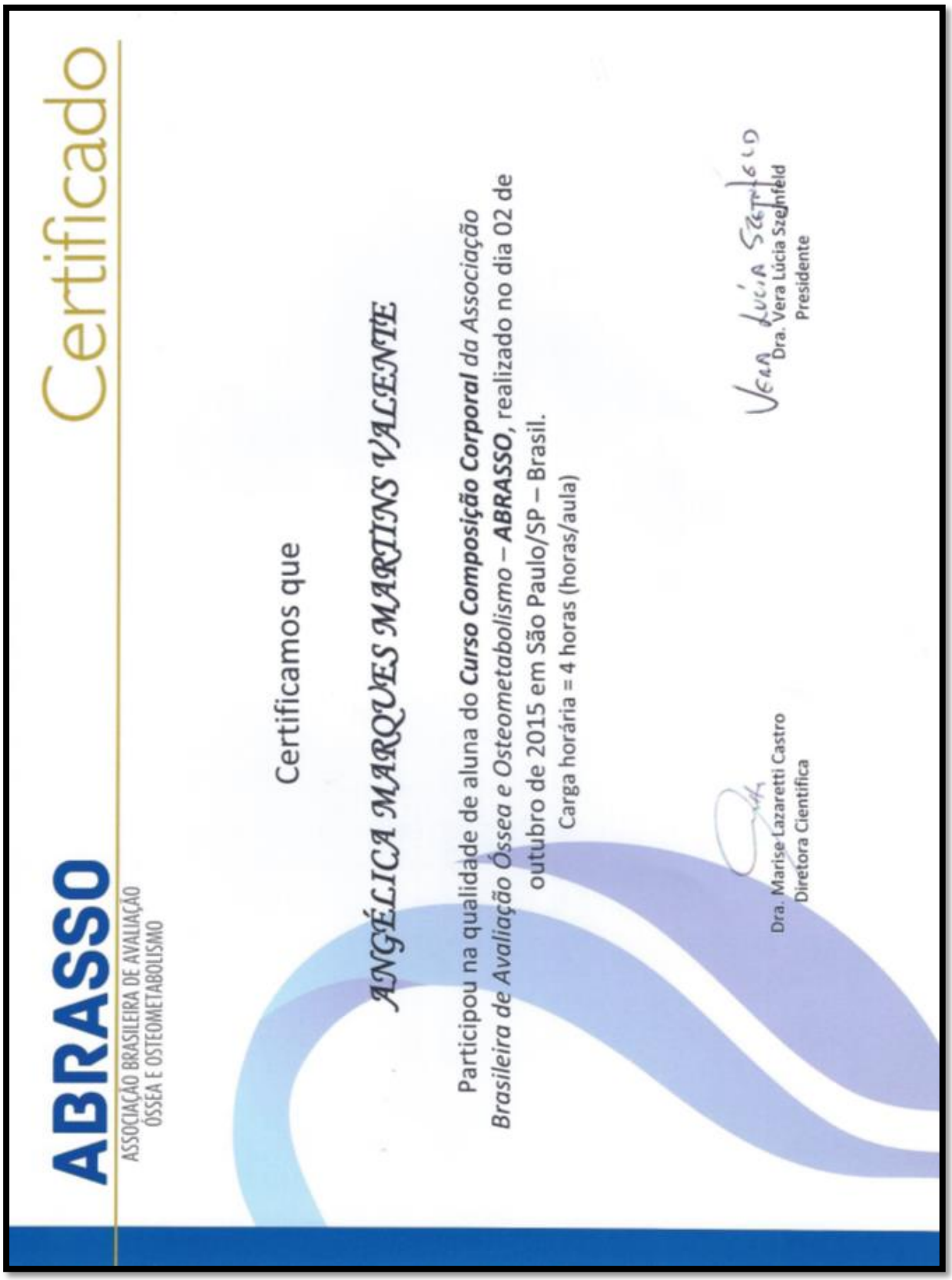
Participou do ***Curso Oficial de Certificação em Densitometria Óssea*** *da Associação Brasileira de Avaliação Óssea e Osteometabolismo –* ***ABRASSO*** */ Colégio Brasileiro de Radiologia Diagnóstico por Imagem –* ***CBR,*** realizado nos dias 24 e 25 de fevereiro de 2018 em São Paulo/SP – Brasil, obtendo aproveitamento no exame de 76%.

Certificado Válido até 25/02/2020

Dr. Francisco José Albuquerque de Paula
Diretor Científico

Dra. Marise Lazaretti Castro
Presidente

<u>Attachment 10</u>: Certificate "Curso de Composição Corporal por DXA – ABRASSO", 2015.



ABRASSO

ASSOCIAÇÃO BRASILEIRA DE AVALIAÇÃO ÓSSEA E OSTEOMETABOLISMO

Certificado

Certificamos que

ANGÉLICA MARQUES MARTINS VALENTE

Participou na qualidade de aluna do ***Curso Composição Corporal*** *da Associação Brasileira de Avaliação Óssea e Osteometabolismo* – ***ABRASSO***, realizado no dia 02 de outubro de 2015 em São Paulo/SP – Brasil.

Carga horária = 4 horas (horas/aula)

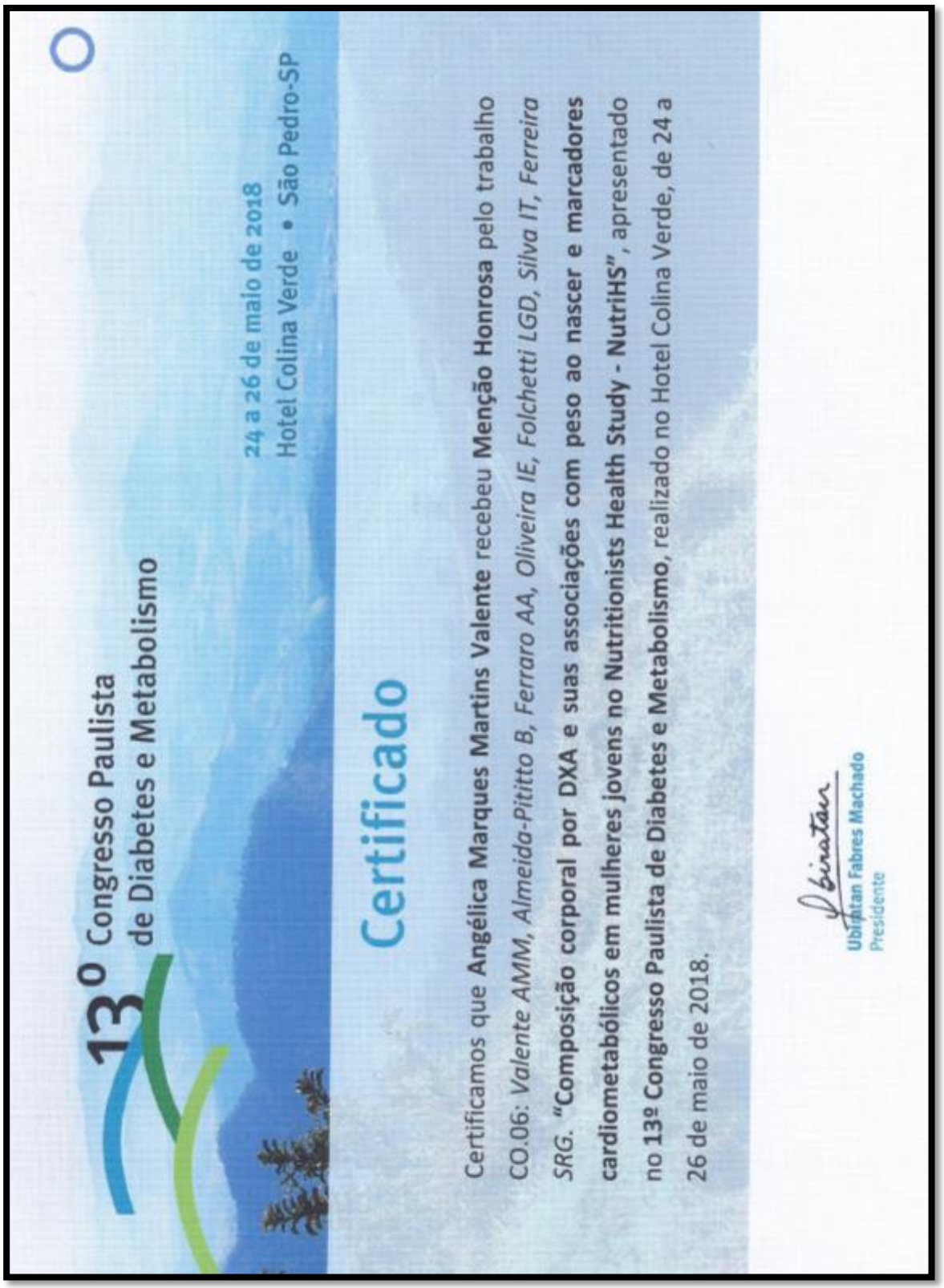
Dra. Marise Lazaretti Castro
Diretora Científica

Vera Lúcia Szejnfeld
Dra. Vera Lúcia Szejnfeld
Presidente

<u>Attachment 11</u>: Award certificates

<u>Attachment 11.1</u>: Award certificate - PRÊMIO DE MELHOR TEMA LIVRE EM ENDOCRINOLOGIA CLÍNICA, no XII COPEM – Congresso Paulista de Endocrinologia e Metabologia, 2017.

<u>Attachment 11.2</u>: Award certificate - Mensão Honrosa no 13º Congresso Paulista de Diabetes e Metabolismo, 2018.



13º Congresso Paulista de Diabetes e Metabolismo

24 a 26 de maio de 2018
Hotel Colina Verde • São Pedro-SP

Certificado

Certificamos que **Angélica Marques Martins Valente** recebeu **Menção Honrosa** pelo trabalho CO.06: *Valente AMM, Almeida-Pititto B, Ferraro AA, Oliveira IE, Folchetti LGD, Silva IT, Ferreira SRG.* **"Composição corporal por DXA e suas associações com peso ao nascer e marcadores cardiometabólicos em mulheres jovens no Nutritionists Health Study - NutriHS"**, apresentado no **13º Congresso Paulista de Diabetes e Metabolismo**, realizado no Hotel Colina Verde, de 24 a 26 de maio de 2018.

Ubiratan Fabres Machado
Presidente

<u>Attachment 11.3</u>: Award certificate - Segundo lugar no prêmio Antônio Carlos Araujo de Souza (MELHOR TRABALHO EM DENSITOMETRIA ÓSSEA) durante o 8º BRADOO, Congresso Brasileiro de Densitometria, Osteoporose e Osteometabolismo, 2018.

CURRICULUM LATTES

PhD CANDIDATE

Angélica Marques Martins Valente

- Endereço para acessar este CV: http://lattes.cnpq.br/1520356428507451
- Última atualização do currículo em 15/12/2018

Possui graduação em Medicina pela Faculdade de Medicina do ABC (1998), Residência Médica em Clínica Médica pela Faculdade de Medicina do ABC (2001) e Mestrado em Medicina (Endocrinologia Clínica) pela Universidade Federal de São Paulo (2005). Atualmente está finalizando seu Doutorado em Epidemiologia pela Faculdade de Saúde Pública da Universidade de São Paulo (FSP-USP). Possui experiência na área de Medicina, com ênfase em Endocrinologia/Clínica Médica e Epidemiologia das Doenças Crônicas não Transmissíveis, atuando principalmente nos seguintes temas: Composição Corporal por Densitometria e Densitometria Óssea, Origens Desenvolvimentistas da Saúde e da Doença (DOHaD), Fatores de Risco Cardiometabólicos e Síndrome Metabólica (Diabetes Mellitus e Obesidade). Possui experiência na área de Pesquisa Clínica como Sub-investigadora e na área de Estatísticas de Saúde com os Programas Estatísticos STATA e R. **(Texto informado pelo autor)**

Graduation at Medicina from Faculdade de Medicina do ABC (1998) and Master's at Endocrinology from Universidade Federal de São Paulo (2005). Has experience in Medicine, focusing on Endocrinology and Epidemiology, acting on the following subjects: body composition and bone densitometry, DOHaD, cardiometabolic risk factors and metabolic syndrome (diabetes and obesity) **(Text informed by the author)**

Identificação

Nome

Angélica Marques Martins Valente

Nome em citações bibliográficas

VALENTE, A. M. M.

Endereço

Endereço Profissional

Universidade de São Paulo. Faculdade de Saúde Pública
Avenida Dr Arnaldo, 715
Pacaembu
01246904 - São Paulo, SP - Brasil
Telefone: (011) 30617870
e-mail: angelicammvalente@uol.com.br

COUNSELOR

Sandra Roberta Gouvea Ferreira Vivolo

Bolsista de Produtividade em Pesquisa do CNPq - Nível 1B

- Endereço para acessar este CV: http://lattes.cnpq.br/6633883139386818
- Última atualização do currículo em 11/12/2018

Possui graduação em Medicina pela Pontifícia Universidade Católica de Campinas (1981), mestrado em Medicina (Endocrinologia Clínica) pela Universidade Federal de São Paulo (1986) e doutorado em Medicina (Endocrinologia Clínica) pela Universidade Federal de São Paulo (1988). É professora titular na Faculdade de Saúde Pública da Universidade de São Paulo e ocupa atualmente o cargo de Chefe do Departamento de Epidemiologia desta Faculdade. Já Tem bolsa de produtividade em pesquisa do CNPq, nível 1-B. Tem experiência na área de Medicina, com ênfase em Epidemiologia e Nutrição, atuando principalmente nos seguintes temas: diabetes mellitus, síndrome metabólica, hipertensão arterial, obesidade, nutrição e microbiota intestinal. **(Texto informado pelo autor)**

Graduated in Medicine by the Pontifícia Universidade Católica de Campinas (1981), master's at Endocrinology Division, Universidade Federal de São Paulo (1986) and PhD at Endocrinology Division, Universidade Federal de São Paulo (1988). She is full professor at the School of Public Health of the University of Sao Paulo. Currently, she is the the head of the Epidemiology Department at the same School. She has experience in Medicine, focusing on Epidemiology and Nutrition, working on diabetes mellitus, metabolic syndrome, hypertension, obesity, nutrition and gut microbiota. **(Text informed by the author)**

Identificação

Nome

Sandra Roberta Gouvea Ferreira Vivolo

Nome em citações bibliográficas

FERREIRA, S. R. G.;Ferreira, Sandra R.;Ferreira, Sandra;Ferreira, Sandra R.G.;Ferreira, Sandra Roberta G.;Ferreira Vivolo, SRG;FERREIRA, Sandra Roberta Gouveia;FERREIRA, Sandra Roberta Gouvea;FERREIRA, Sandra R G;Ferreira, S.R.G.;FERREIRA, SANDRA R;FERREIRA, SANDRA R. G.;FERREIRA, SANDRA RG;VÍVOLO, SANDRA ROBERTA GOUVEA FERREIRA;Sandra Roberta Gouvea Ferreira

Endereço

Endereço Profissional

Universidade de São Paulo, Faculdade de Saúde Pública.
Avenida Dr. Arnaldo, 715
Paraiso
01246-904 - Sao Paulo, SP - Brasil
Telefone: (11) 30617705
Ramal: 218
Fax: (11) 30616601

CO-ADVISER

Bianca de Almeida Pititto

- Endereço para acessar este CV: http://lattes.cnpq.br/8433932854107690
- Última atualização do currículo em 11/12/2018

Possui formação em Clínica Médica, mestrado em fatores de risco cardiovascular pela Endocrinologia da Universidade Federal de São Paulo - UNIFESP (2003) e doutorado em intervenção para prevenção de diabetes mellitus e impacto em fatores de risco cardiovascular pela Faculdade de Saúde Pública-USP (2009), tendo sido "visiting student" no MRC Epidemiology Unit of Cambridge University-UK (2007). Finalizou pós-doutorado pela Faculdade de Saúde Pública - USP (2013), estudando fatores de risco cardiovascular não tradicionais em coorte de estudo epidemiológico, ELSA-SP. Curso de pós-graduação Latu Senso em Geriatria na UNIFESP e na USP. É médica concursada do Departamento de Medicina Preventiva-UNIFESP, atuando na graduação e residência médica e orientação de pós-graduação pelo Programa de Pós-Graduação em Endocrinologia Clínica, na área de epidemiologia das doenças crônicas não transmissíveis e fatores de risco cardiometabólicos (disglicemia, dyslipidemia, hipertensão, obesidade). Áreas de atuação: epidemiologia, diabetes mellitus, obesidade, doenças crônicas não transmissíveis, fatores de risco cardiovascular, prevenção e epidemiologia do ciclo vital (eventos precoces da vida). **(Texto informado pelo autor)**

Master's at Endocrinology from Universidade Federal de São Paulo (2003) and doctorate at Collective Health from Universidade de São Paulo (2009). Has experience in Medicine, acting on the following subjects: cardiovascular risk factors, diabetes mellitus, epidemiologia, metabolic syndrome and body fat distribution. **(Text informed by the author)**

Identificação

Nome

Bianca de Almeida Pititto

Nome em citações bibliográficas

Almeida-Pititto B;Almeida-Pititto, Bianca de;Almeida B;Almeida-Pititto Bianca;B. Almeida-Pititto;ALMEIDA-PITITTO, B.;ALMEIDA-PITITTO, BIANCA;DE ALMEIDA-PITITTO, BIANCA;DE ALMEIDA-PITITTO, B.

Endereço

Endereço Profissional

Universidade Federal de São Paulo, Departamento de Medicina Preventiva.
Rua Borges Lagoa - de 1233 ao fim - lado ímpar
Vila Clementino
04038034 - São Paulo, SP - Brasil