U.PORTO

MESTRADO INTEGRADO EM MEDICINA

Bone metabolism assessment in an adolescent's population with anorexia nervosa: a cross-sectional study of a tertiary care hospital

Maria Inês Lourenço Cardia

M

2024

Bone metabolism assessment in an adolescent's population with anorexia nervosa: a cross-sectional study of a tertiary care hospital

ARTIGO ORIGINAL

Dissertação de Candidatura ao Grau de Mestre em Medicina
Submetida ao Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto

Estudante: Maria Inês Lourenço Cardia
Aluna do 6º ano do Mestrado Integrado em Medicina
Afiliação: Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto
Endereço eletrónico: inescardia99@gmail.com/up201704510@up.pt

Orientadora: Dr.ª Helena Maria Castro Moura Ferreira Mansilha
Assistente Graduada de Pediatria
Serviço de Pediatria
Centro Materno Infantil do Norte da Unidade Local de Saúde de Santo António
Colaboradora Externa do Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto

Coorientadora: Professora Doutora Esmeralda Emília Gomes Martins
Assistente Graduada Sénior de Pediatria
Serviço de Pediatria
Centro Materno Infantil do Norte da Unidade Local de Saúde de Santo António
Professora Catedrática Convidada, Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto

Estudante

Orientadora

Coorientadora

Porto, Maio 2024

Declaration of Scientific Integrity

I, Maria Inês Lourenço Cardia, student number 201704510 of the Integrated Master's Degree in Medicine of the School of Medicine and Biomedical Sciences of the University of Porto, declare that this dissertation is my own and has not been previously used in any other course or curricular units, from this or another institution. References to other authors (statements, ideas, thoughts) scrupulously respect the attribution rules and are duly indicated in the text and bibliographical references, per the referencing rules. I am aware that the practice of plagiarism and self-plagiarism constitutes an academic offence.

Maria Inês Lourenço Cardia

Porto, May 2024

To my family and my outstanding friends,

In the vastness of space and the immensity of time, it is my joy to share a planet and an epoch with you.

Adapted from Carl Sagan's *Cosmos*, 1980

ACKNOWLEDGMENTS

First, my sincere thanks to Doctor Helena Ferreira Mansilha for embracing this project with open arms, for all the guidance and support throughout the year and for the unconditional availability for all my questions, worries and doubts.

I would like to thank Professor Doctor Esmeralda Martins for agreeing to take part in this project.

A special thanks to Ana Gonçalves, Maria Gonçalves and Sara Pires Costa for their help with statistical analysis.

Last but not least, I'm thankful to my mother for all the help with the English language.

RESUMO

Contexto: A Anorexia Nervosa (AN) é uma doença psiquiátrica potencialmente fatal que geralmente tem início na adolescência. Um peso corporal inferior ao normal é mantido principalmente através do jejum, levando à desnutrição e resultando em complicações somáticas, como o hipogonadismo hipogonadotrófico. O pico de massa óssea é alcançado durante a adolescência e início da idade adulta, altura em que o estrogénio atua como estímulo para a formação óssea, na presença de energia e substrato. O aumento da massa muscular induzido hormonalmente melhora, igualmente, a resistência óssea. Nestes doentes, a deficiência de estrogénio e a diminuição do peso corporal e da massa muscular resultam em baixa densidade mineral óssea (BMD), aumentando o risco de fratura. Mesmo após a recuperação da AN, a baixa BMD persiste, causando incapacidade permanente. Atualmente, o tratamento mais eficaz para esta complicação consiste na melhoria do estado nutricional.

Objetivos: Analisar a saúde óssea de uma população de adolescentes com AN seguidos pelo Grupo Multidisciplinar de Perturbações do Comportamento Alimentar na Adolescência de um hospital terciário.

Materiais e Métodos: Foram recolhidos parâmetros clínicos e analíticos (incluindo cálcio, fósforo, vitamina D, fosfatase alcalina fração óssea, paratormona, propeptido aminoterminal do procolagénio tipo I) de 120 adolescentes com diagnóstico de AN. Alguns doentes realizaram uma Densitometria Óssea (DXA) e foram divididos em grupos de acordo com o mínimo Z-score obtido na DXA.

Resultados: A idade e a idade pré-mórbida em doentes com envolvimento ósseo foram estatisticamente significativamente superiores do que em pacientes com BMD normal, com valor de $p < 0.001$ e 0.003, respetivamente. O Z-score do peso e o Z-score do peso pré-morbido foram estatisticamente significativamente inferiores em doentes com envolvimento ósseo ($p=0.018$ e $p=0.037$, respetivamente). O Z-score da estatura foi estatisticamente significativamente inferior em doentes com baixa BMD ($p=0.042$). A massa livre de gordura foi estatisticamente significativamente inferior em pacientes com baixa BMD ($p=0.022$). Não foram encontradas associações estatisticamente significativas entre a BMD obtida pela DXA e duração da doença, duração da amenorreia e parâmetros séricos do metabolismo ósseo.

Discussão/Conclusão: Na nossa população de adolescentes com AN, a saúde óssea parece estar comprometida. A duração da amenorreia não parece estar associada à saúde óssea. A gravidade e/ou estado prolongado da desnutrição, particularmente a perda de massa muscular,

mostraram uma associação estatisticamente significativa com a baixa BMD. Na nossa população, a DXA parece ser o melhor método para determinar a gravidade e evolução da saúde óssea.

Palavras-chave: Anorexia Nervosa; Amenorreia; Adolescente; Doenças Ósseas, Metabólicas

ABSTRACT

Background: Anorexia Nervosa (AN) is a potentially life-threatening psychiatric disorder that typically begins in adolescence. Patients maintain a below-normal body weight mainly by starvation, which leads to malnutrition, resulting in somatic complications such as hypogonadotropic hypogonadism. Peak bone mass is achieved during adolescence and early adulthood, in which oestrogen is the stimuli for bone formation, in the presence of energy and substrate. Hormonal-induced increase in muscle mass also improves bone strength. In disease scenarios, oestrogen deficiency, lower body weight and lower muscle mass result in low bone mineral density (BMD), increasing fracture risk. Even after recovery from AN, low BMD persists, causing permanent disability. Presently, the most effective treatment for this complication is improving nutritional status.

Objectives: To analyse bone health in a population of adolescents with AN monitored by the Adolescent Eating Disorders Multidisciplinary Group of a specialized tertiary hospital.

Materials and Methods: Clinical and analytical parameters (including calcium, phosphorus, vitamin D, bone-specific alkaline phosphatase and procollagen type 1 N-terminal propeptide) were collected from 120 adolescents diagnosed with AN. Some patients underwent a Dual-Energy X-ray Absorptiometry (DXA) and were split into groups according to minimum DXA Z-score.

Results: Age and pre-morbid age in patients with bone impairment were statistically significantly higher than in patients with normal BMD with a p-value <0.001 and 0.003, respectively. Weight Z-score and pre-morbid weight Z-score were statistically significantly lower in patients with bone impairment (p=0.018 and p=0.037, respectively). Height Z-score was statistically significantly lower in patients with low BMD (p=0.042). Fat-free mass was statistically significantly lower in patients with low BMD (p=0.022). No statistically significant associations were found between BMD assessed by DXA scan and duration of illness, duration of amenorrhea and serum bone metabolism parameters.

Discussion/Conclusion: In our population of adolescents with AN, bone health appears to be compromised. Length of amenorrhea was not found to be associated with bone health. Severity and/or a prolonged state of malnutrition, particularly loss of muscle mass, showed a statistically significant association with low BMD. In our cohort, the DXA scan seems the best method for determining the severity and evolution of bone health.

Key-Words: Anorexia Nervosa; Amenorrhea; Adolescent; Bone Diseases, Metabolic

ABBREVIATION'S LIST

AN – Anorexia Nervosa

ANOVA – Analysis of Variance

ATP – Adenosine Triphosphate

BALP – Bone-specific Alkaline Phosphatase

BIA – Bioimpedance Analysis

BMD – Bone Mineral Density

BMI – Body Mass Index

CMIN – Centro Materno-Infantil do Norte

DSM-5-TR - Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition, Text Revision

DXA - Dual-energy X-ray Absorptiometry

I-BMD – Impaired Bone Mineral Density

Kg – Kilogram

L-BMD – Low Bone Mineral Density

LH - Luteinizing Hormone

N-BMD – Normal Bone Mineral Density

PTH - Parathyroid Hormone

P1NP - Procollagen Type 1 N-terminal Propeptide

RL-BMD – at Risk for Low Bone Mineral Density

SD – Standard Deviation

TBS – Trabecular Bone Score

WHO – World Health Organization

Z-sc – Z-score

INDEX

TABLE'S LIST

INTRODUCTION

Anorexia Nervosa (AN) is defined by the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition, Text Revision (DSM-5-TR) as a "persistent restriction of energy intake leading to significantly low body weight with either intense fear of gaining weight or becoming fat, or persistent behaviour that interferes with weight gain, with disturbance in the way one's body weight or shape is experienced, or denial of the seriousness of the current low body weight"[1]. This is a potentially life-threatening severe psychiatric disorder with an increasing prevalence[2]. Although AN can develop at any age, it typically begins in adolescence[3] and currently affects around 0.9% adolescents[4,5] in a proportion of 10:1 females to males[6]. This condition has the highest mortality rate of all psychiatric disorders[7].

These patients maintain a below-normal body weight mainly by starvation, with inadequate supply of nutrients and energy to meet the body's needs, which in turn leads to malnutrition[6,8,9]. Chronic malnutrition results in global somatic complications and decreased quality of life[6,10].

The body's low-energy state induces endocrine disturbances, such as hypogonadotropic hypogonadism – characterised by low amplitude Luteinizing Hormone (LH) pulsatility with low oestrogen levels, similar to prepubertal girls' patterns – inducing a delay in puberty and primary or secondary amenorrhea, with low oestrogen levels[3,8,10,11].

Peak bone mass is achieved throughout adolescence and early adulthood[8,12]. Sexual hormones, particularly oestrogen, have an essential function in maintaining skeletal homeostasis by engaging in bone production and reabsorption processes[3]. Besides the hormonal stimuli for bone formation, substrate is also needed for the bone remodelling process, namely energy for adenosine triphosphate (ATP) production[13], amino acids[13], calcium and phosphate ions[3,13], and vitamin D and K[3,11,13,14].

Furthermore, the mechanostat theory states that mechanical loads are what stimulate the growth of bone strength[15]. Sex hormone-induced muscle mass gains during puberty, physical growth spurts and physical exercise lead to improvements in bone strength. However, in AN, this is not observed, as oestrogen deficiency causes a deficit in trabecular bone, and low levels of body weight and muscle mass lessen the mechanical pressure on the bone, impacting negatively osteogenesis in these patients[15].

Therefore, AN is associated with a low bone mineral density (BMD), being more significant in adolescent than adults[9]; the majority (52%) of these patients have Dual-energy X-ray Absorptiometry (DXA) Z-scores lower than –1[11], and 46% manifest an increased fracture risk

persisting up to 40 years after diagnosis in 46%[14]. Low BMD can continue for decades after illness resolution, causing permanent disability[5].

Thus, considering the aforementioned characteristics, it is pivotal to focus on treatment strategies. It is known that improving nutritional status is strongly associated with a significant increase in BMD in the long term[16]. Additionally, oestrogen replacement therapy and bisphosphonates are, amongst others, currently being studied as a possible treatment option[17,18]. However, despite a plethora of trials being conducted to minimize and treat bone disease in AN, these therapies have non-negligible adverse reactions and did not prove to be a successful strategy for low BMD[7,17,19].

In light of the aforementioned, we intend to analyse the bone health of a population of adolescents with AN monitored by the Adolescent Eating Disorders Multidisciplinary Group of a specialized tertiary hospital.

METHODS

This cross-sectional prospective study was conducted in the Adolescent Eating Disorder Multidisciplinary Group, involving the Adolescent Psychiatric Department and the Paediatric Department of Centro Materno-Infantil do Norte (CMIN), a specialised tertiary care university hospital. From November 2023 to March 2024, 1170 outpatients were performed. Of those, all patients in paediatric age (<18 years) diagnosed with AN meeting DSM-5-TR (2022) criteria were invited to participate in the study. Patients with other primary eating disorders' diagnoses were not included. 120 patients met these criteria.

Weight was measured in kilograms (kg) using a certified digital scale. Height was measured in centimetres (cm) with a certified stadiometer. Body Mass Index (BMI) was calculated using weight and height according to the following formula: $BMI = \frac{weigh}{heigh^{2}}$. Posteriorly, weight, height and BMI Z-scores were individually calculated using World Health Organization (WHO) Child Growth Standards, adjusted to age in months. Fat mass and fat-free mass were measured in kg using InBody 270®, performing a Bioelectrical Impedance Analysis (BIA).

Age of menarche, duration of amenorrhea, duration of illness and pre-morbid weight were registered.

To assess bone metabolism, parameters regarding bone health were collected from a blood sample, including calcium[13], phosphorus[13], 25-hydroxy vitamin D (25OHD)[13], bone-specific alkaline phosphatase (BALP)[20] and procollagen type 1 N-terminal propeptide (P1NP)[13,20].

To assess BMD, patients aged over 8 years old and who had either amenorrhea for over 6 months or were males with duration of illness higher than 6 months, underwent a DXA scan. For our analysis, the lowest Z-score was used from either lumbar spine, hip, or whole-body values.

For research purposes, patients were split into 2 groups, according to minimum DXA Z-score: "normal BMD (N-BMD)" consisting of patients with DXA Z-score higher than -1 SD (standard deviation) and "with impaired BMD (I-BMD)", consisting of patients with DXA Z-score lower than -1 SD. The group I-BMD was further split into "low BMD (L-BMD)", with patients with DXA Z-score lower than -2[21] and "at risk for low BMD (RL-BMD)", with patients with DXA Z-score between -1 and -2.

For the descriptive statistics, absolute frequencies (N) and relative frequencies (%) were used for categorical variables, and means and standard deviations (SD) were used in continuous variables. Nominal variables were compared using Qui-Square test. Clinical data variables and bone metabolism parameters were compared according to bone disease variables using t-test for independent samples and one-way Analysis of Variance (ANOVA), considering the result of the

statistic test significant for a p value <0.05. Statistical analysis is performed using IBM SPSS version 29.0.0.0 program.

This study was conducted under local ethics committee approval (2023.265(227-DEFI/217-CE)).

RESULTS

Sample Characteristics

Sample characteristics for the whole study group are presented in Tables I and II. From the 1170 Paediatric Nutrition outpatients performed during the period of study, 120 adolescents met our study's criteria. Out of the 120 patients, 111 were female (92.5%) and the mean age was 16.05 (SD=1.71) years. Mean pre-morbid age was 13.78 (SD=1.57) years. Mean duration of illness was 2.26 (SD=1.17) years, ranging from 0.09 to 5.66 years. Mean pre-morbid weight Z-score was 0.3163 (SD=0.96287) Z-sc and mean weight Z-score was -1.0796 (SD=1.46849) Z-sc. Mean height Z-score was 0.0884 (SD=0.90468) Z-sc. Mean BMI Z-score was -1.19 (SD=1.506) Z-sc. Mean fat mass was 9.208 (SD=5.8237) kg. Most patients, 110 (92.4%), reached puberty. Of those, excluding male participants, 59 (56.7%) patients were presently amenorrheic, with a mean duration of amenorrhea of 18.56 (SD=10.871) months.

Regarding bone metabolism, the following mean serum parameters were registered: calcium 2.4605 (SD=0.26396) mg/dL; phosphorus 1.2535 (SD=0.14074) mg/dL; PTH 34.115 (SD=15.1470) pg/mL; BALP 20.974 (SD=20.7105) U/L; vitamin D 59.598 (SD=23.6516) ng/mL and P1NP 90.5800 (SD=109.21233) mcg/L.

A DXA scan was performed on 51 (42.5%) patients, with an average DXA minimum Z-score of -1.2667 (SD=1.25836) Z-sc. Thus, 31 (60.8%) patients had bone impairment. Of those, 16 (51.6%) had low BMD.

Amenorrhea comparisons

Weight Z-score's mean was statistically significantly lower in the amenorrheic group, compared to the non-amenorrheic group (-1.591 Z-sc *vs*. -0.598 Z-sc; p<0.001). BMI Z-score mean was also statistically significantly lower in the amenorrheic group (-1.710 Z-sc *vs*. -0.572 Z-sc; p<0.001), as well as total body fat in kg mean (7.536 kg *vs*. 12.518 kg; p<0.001). There were no statistically significant associations between presence or absence of amenorrhea and age, pre-morbid age, height, fat-free mass and duration of illness (Table III). No statistically significant associations were found between presence or absence of amenorrhea and bone metabolism parameters (Table IV).

Normal (N-BMD) *vs*. Impaired bone (I-BMD)

There was no statistically significant difference found between presence (I.BMD) or absence of bone impairment (N-BMD) and presence or absence of amenorrhea (qui-square test value of 0.086).

The mean current age was statistically significantly higher in the I-BMD group, compared to the N-BMD group (17.0755 years *vs*. 15.2640 years; p<0.001). The mean pre-morbid age was statistically significantly higher in the I-BMD group compared to the N-BMD group (14.4604 years *vs*. 13.0950 years; p<0.001). Mean weight Z-score was statistically significantly lower in the I-BMD group (-1.7148 Z-sc *vs*. -0.9460 Z-sc; p=0.032). Mean pre-morbid weight Z-score was statistically significantly lower in the I-BMD group (-0.1243 Z-sc *vs*. 0.2565 Z-sc; p=0.044). There were no statistically significant associations found between N-BMD and I-BMD groups regarding duration of illness, height Z-score, BMI Z-score, fat mass, fat-free mass or duration of amenorrhea (Table V). There were no statistically significant findings between the two groups and serum bone metabolism parameters (calcium, phosphorus, PTH, BALP, vitamin D and P1NP) (Table VI).

Degree of bone impairment (N-BMD *vs*. RL-BMD *vs*. L-BMD)

When comparing clinical variables with degree of bone impairment, mean age was statistically significantly different in all three groups (15.2640 years *vs*. 16.8767 years *vs*. 17.2619 years; p<0.001), where the N-BMD group's mean age was lower than the RL-BMD group, which was lower than the L-BMD group. Mean pre-morbid age was statistically significantly different in the three groups as well (13.0950 years *vs.* 14.5529 years *vs*. 14.3679 years; p=0.003). Mean weight Z-score was statistically significantly different in the three groups (-0.9460 Z-sc *vs*. –1.1600 Z-sc *vs*. –2.2350 Z-sc; p=0.018), being the lowest in the L-BMD group. Mean pre-morbid weight Z-score was also statistically significantly different in the three groups (0.2565 Z-sc *vs*. 0.1457 Z-sc *vs*. –0.3943Z-sc; p=0.037).

There were no statistically significant differences between degree of bone impairment and duration of illness, height Z-score, BMI Z-score, fat mass, fat-free mass and duration of amenorrhea (Table VII). There were no statistically significant variations between the three groups and calcium, phosphorus, PTH, BALP, vitamin D and P1NP (Table VIII).

At risk of low BMD *vs*. Low BMD

Within the I-BMD group, we found that the mean weight Z-score of the L-BMD patients was statistically significantly lower than the mean weight Z-score of the RL-BMD patients (-2.2350 Z-sc *vs*. –1.1600 Z-sc; p=0.017). The mean pre-morbid weight Z-score was statistically significantly lower in the L-BMD group, compared to the RL-BMD group (-0.3943 Z-sc *vs*. 0.1457 Z-sc; p=0.019). The mean height Z-score of the L-BMD group was statistically significantly lower when compared to the mean height Z-score of the RL-BMD group (-0.5787 Z-sc *vs*. 0.0227 Z-sc; p=0.042). We also found

that the mean fat-free mass in kg was significantly lower in the L-BMD group (18.169 kg *vs*. 20.140 kg; p=0.022).

There were no statistically significant differences between RL-BMD and L-BMD groups regarding age, pre-morbid age, duration of illness, BMI, fat mass and duration of amenorrhea (Table IX).

There were no statistically significant differences between these two groups regarding serum bone metabolism parameters (Table X).

Normal and at risk of low BMD (N-BMD and RL-BMD) *vs*. low BMD (L-BMD)

When comparing the N-BMD and RL-BMD groups with the L-BMD group, we concluded that the mean age in the L-BMD group was statistically significantly higher (15.9551 years *vs*. 17.2619 years; p=0.007). The mean weight Z-score in the L-BMD group was statistically significantly lower when compared to the N-BMD and RL-BMD group (-1.0377 Z-sc *vs*. –2.2350 Z-sc; p=0.005). The mean pre-morbid weight Z-score was statistically significantly lower in the L-BMD group (0.2109 Z-sc *vs*. –0.3943 Z-sc; p=0.011). Mean height Z-score was also statistically significantly lower in the L-BMD group (0.0423 Z-sc *vs*. –0.5787 Z-sc; p=0.021). Mean BMI Z-score in the L-BMD group was statistically significantly lower as well (-1.2637 Z-sc *vs*. –2.1231 Z-sc; p=0.029). Mean fat-free mass in kg in the L-BMD group was statistically significantly lower (20.174 kg *vs*. 18.169 kg; p=0.047).

No statistically significant differences were found when comparing these groups regarding pre-morbid age, duration of illness, fat mass and duration of amenorrhea (Table XI).

There were no statistically significant differences found between N-BMD+RL-BMD group and L-BMD group in bone metabolism parameters (Table XII).

DISCUSSION

Globally, AN has a prevalence of almost 1%[4,5], of which 10% are males[6,11]. Similarly, in our cohort, the percentage of male participants is 7.5%, supporting the fact that our cohort may be representative of all patients with this condition.

Knowing that the youngest patient included in our study was 10 years and 7 months old, and that the patient with the earliest pre-morbid age was 9 years old at onset of symptoms, we can affirm that our study consists of a particularly young population (with 9% prepuberal patients), not frequent in literature. However, it also depicts the alarming reality that AN can present in extremely early ages, before puberty onset.

In our population, 56.7% of female patients were amenorrheic, a low fraction compared to the one's described in other studies[4,22]. Nevertheless, due to the cross-sectional nature of our study, patients present themselves in different stages of disease and a lot of female patients had, at the time of our study, resumed menses.

In our adolescents' population diagnosed with AN, the weight and BMI Z-scores, as well as fat mass in kg, were lower in amenorrhoeic patients. These results corroborate the findings encountered by Winkler *et al.*, stating that fat percentage and BMI were positively associated with menses[23]. In this disease, nutritional status can be expressed by lower weight, BMI and fat mass[9], and malnutrition affects multiple endocrine axes, particularly hypothalamic-pituitary-gonadal axis, inducing very low amplitude LH pulses or a sleep entrained pattern of pulsatility[8]. This change is the most likely cause of hypothalamic amenorrhea observed in these patients, which leads to hypoestrogenism[8]. Therefore, it's expected that weight, BMI and fat mass are lower in the amenorrhoeic patients[24], as found in our population.

No statistically significant association was observed between duration of illness and bone impairment. This finding differs from other results described in literature, where longer duration of illness appears to reduce BMD[4,6,25,26]. However, most studies performed in populations with AN consist of adults rather than adolescents who did not start peak bone mass development, including early childhood (minimum age of 10 years), which may explain this difference. Donaldson *et al.,* who studied a population consisting of 57 adolescent girls, described no association between duration of illness and bone impairment[27]. Robinson *et al.* run a meta-analysis, including 27 studies performed from 1990 to 2014, with a population of female participants aging from 15.9 to 34.3 years old[28], and found no statistically significant association between both these variables[28], such as found in our population.

Overall, current age and pre-morbid age in I-BMD patients were significantly higher than in N-BMD patients. To our knowledge, no other study describes this positive association. The fact that peak

bone mineral accretion occurs in late adolescence and early adulthood might help to explain this finding[12,29]. This information underlines that, in early stages of life, bone mass is yet to start to increase, and, as a result, the range and difference in DXA Z-score values are smaller.

Patients with bone impairment had a lower weight Z-score. This result has already been reported in the literature by Donaldson *et al.,* who assessed bone health using trabecular bone score (TBS) rather than BMD reported by DXA[27] and found that TBS correlates to BMD, and was lower in adolescents with lower weight in kg[27]. Ostrowska *et al.* studied a population of 75 adolescent girls and, similar to us, grouped the patients according to DXA Z-scores[30], showing, amongst other findings, that DXA Z-scores were lower in patients with lower weight in kg[30]. This association might be attributed to patients' decreased energy levels due to their low body weight.[31]

In addition, we discovered — to our knowledge, a result not previously reported— that patients with bone impairment had lower pre-morbid weight Z-scores. The idea that adolescents with a lower pre-morbid weight are more likely to develop severe malnutrition, and that this energy deficit condition implies a quicker compromise of bone health may help to explain this relation.

Patients with low BMD had lower height Z-scores, compared to those who have higher BMD, as Donaldson *et al.* had also previously demonstrated[27]. However, many studies performed in this field do not include height in their study variables, which may justify the lack of literature available describing this association[4,32]. This association shows that persistent malnutrition translates not only in decreased weight but also in a compromised height, where BMD is not enhanced with normal adolescent hormonal stimuli and therefore leads to a diminished acquisition of bone mass[33].

Patients within L-BMD group had lower fat-free mass when compared to the ones within N-BMD and RL-BMD groups. This is an already known association, described by Donaldson *et al*[27]. Lu *et al.* reached the same conclusion, regarding a population composed of female patients aged 15 to 40 years with functional hypothalamic amenorrhea (including patients with female athlete triad as well as AN)[32]. Bemer et al. found this association comparing DXA Z-scores in their population of 97 extremely malnourished female inpatients with AN, aged equal or higher than 16 years old[6]. Svedlund et al. also reported this association in a prospective study consisting of a small population with 22 adult patients with AN[22]. The chronic malnutrition these patients undergo leads, firstly, to a decrease in fat mass and, as the disease progresses, to a decrease in muscle mass as well. This association also emphasises the role of muscle mass in raising mechanical stress, consequently boosting bone strength, and supporting the mechanostat hypothesis[15].

There were no statistically significant associations found between BMD assessed by DXA scan and serum bone metabolism parameters. This lack of significant associations may be due to the high efficiency of phosphorus and calcium homeostasis. This way, we suggest that the DXA scan, albeit

its high cost, should not be replaced with another analytical blood analysis, given the lack of association found between them and DXA results assessing and monitoring bone health.

Even though AN is a psychiatric condition, adolescents with this diagnosis should receive specialised and experienced interdisciplinary follow-up, as it causes a serious compromise to one's organic and physical health, namely bone health. Since bone mass reaches its peak in adolescence/late teens, it is particularly important to monitor this serious issue during these years as it can completely and permanently impair bone health.

Our study's findings suggest that adolescents who have not only extended amenorrhea, but also lower fat-free mass as determined by BIA, a lower Z-score BMI, or who are older and most at risk of not attaining peak bone mass should undergo a DXA scan, in order to assess BMD.

Strengths

We combined a large patient base from a single medical facility, monitored by a skilled multidisciplinary team with a paediatric and paedopsychiatric approach, guaranteeing a consistent and protocolary treatment for these adolescents. It is, therefore, anticipated that our findings may be reproducible.

Our cohort comprised prepubertal patients of early age. This trait is seen in just a few studies, which mostly include adults and adolescents over the age of 15.

Limitations and Future Work

This study is a cross-sectional cohort study where associations between clinical, analytical and imaging variables and recovery of nutritional status of the individual during follow-up were not possible. Another limiting factor is the fact that not every patient met the criteria to perform a DXA scan, which reduced the sample size for some variables.

We suggest that this study is reproduced within other cohorts of patients to have a more comprehensive view of bone health in this population. In future studies, after weight restoration, it is also apprised to do a DXA scan following nutritional recovery, to monitor bone health and, potentially, BMD recovery. Considering that many adult studies report low BMD in AN patients[5,7,14], it may be crucial to better understand this correlation, acting early to minimise bone damage and highlight the importance of investment in a specialised multidisciplinary follow-up.

CONCLUSION

AN is a highly prevalent psychiatric illness affecting adolescents, entailing major somatic impact, namely in bone health. Our study showed that length of amenorrhea was not found to be associated with bone health. Severity and/or a prolonged stage of malnutrition, particularly loss of muscle mass, showed a statistically significant correlation with low BMD.

A DXA scan was the best examination for determining the severity and evolution of bone health, and analytical parameters did not correlate to impairment of BMD.

The management of these adolescents with a comprehensive and specialised approach is essential to improve the overall prognosis and specifically bone health's.

APPENDIX

Table I – Characteristics of 120 adolescents with anorexia nervosa (continuous variables)

Continuous Variables	n	Mean ± SD	Interval	
			Min	Max
Age (years)	120	16.0547±1.70819	10.58	19.75
Pre-morbid age (years)	117	13.7754±1.57083	9.00	17.75
Duration of illness (years)	117	2.2550±1.16738	0.09	5.66
Pre-morbid weight (Z-score)	115	0.3163±0.96287	-2.88	3.15
Current weight (Z-score)	120	-1.0796±1.46849	-5.32	2.86
Height (Z-score)	120	-0.0884±0.90468	-2.24	3.68
BMI (Z-score)	120	-1.1919±1.50652	-5.07	2.01
Fat mass (kg)	119	9.208±5.8237	0.8	31.5
Fat-free mass (kg)	119	20.379±4.6012	13.2	47.9
Amenorrhea (months)	57	18.56±10.871	3	43
Calcium (mg/dL)	108	2.4605±0.26396	2.08	4.90
Phosphorus (mg/dL)	68	1.2535±0.14074	0.87	1.65
PTH (pg/mL)	39	34.115±15.1470	16.6	108.0
BALP (U/L)	101	20.974±20.7105	5.4	156.0
Vitamin D (ng/mL)	89	59.598±23.6516	23,0	168.0
P1NP (mcg/L)	57	90.5800±109.21233	15.25	607.50
DXA minimum (Z-score)	51	-1.2667±1.25836	-6.00	1.30

Table II – Characteristics of 120 adolescents with anorexia nervosa (categorical variables)

Categorical Variables	n	Number of patients (%)
Sex	120	
Female		111 (92.5%)
Male		9 (7.5%)
Reached puberty	119	
No		9 (7.6%)
Yes		110 (91.7%)
Amenorrhea	104	
No		45 (43.3%)
Yes		59 (56.7%)
Bone impairment	51	
No (N-BMD)		20 (39.2%)
Yes (I-BMD)		31 (60.8%)
Degree of bone impairment	51	
Normal BMD (N-BMD)		20 (39.2%)
At risk of low BMD (RL-BMD)		15 (29.4%)
Low BMD (L-BMD)		16 (31.4%)

Table III – Clinical variables and mean comparison (t-test for independent samples) with presence of amenorrhea (n=104)

Variables	Amenorrhea		p value
	yes	no	
Age (years)	16.2381	16.3951	0.606
Pre-morbid age (years)	14.0542	13.8252	0.441
Duration of illness (years)	2.1791	2.5277	0.146
Pre-morbid weight (Z-score)	0.2184	0.2579	0.844
Current weight (Z-score)	-1.5905	-0.5984	<0.001***
Height (Z-score)	-0.1463	-1.076	0.833
BMI (Z-score)	-1.7100	-0.5722	<0.001***
Fat mass (kg)	7.536	12.518	<0.001***
Fat-free mass (kg)	19.569	20.609	0.121

*significant at p<0.05; **significant at p<0.01; ***significant at p<0.001

Table IV – Bone metabolism parameters and mean comparison (t-test for independent samples) with presence of amenorrhea (n=104)

Variables	Amenorrhea		p value
	yes	no	
Calcium (mg/dL)	2.4567	2.4834	0.341
Phosphorus (mg/dL)	1.2424	1.2695	0.251
PTH (pg/mL)	36.705	32.347	0.198
BALP (U/L)	18.798	18.079	0.404
Vitamin D (ng/mL)	58.156	58.369	0.483
P1NP (mcg/L)	76.2226	73.9320	0.906
DXA minimum Z-score	-1.2812	-1.3647	0.425

*significant at p<0.05; **significant at p<0.01; ***significant at p<0.001

Table V – Clinical variables and mean comparison (t-test for independent samples) with presence of bone impairment (N-BMD vs. I-BMD) (n=51)

N-BMD: Normal bone mineral density

I-BMD: Impaired bone mineral density

Variables	Bone Impairment		p value
	Yes (I-BMD)	No (N-BMD)	
Age (years)	17.0755	15.2640	<0.001***
Pre-morbid age (years)	14.4604	13.0950	<0.001***
Duration of illness (years)	2.6232	2.1690	0.189
Pre-morbid weight (Z-score)	-0.1243	0.2565	0.044*
Current weight (Z-score)	-1.7148	-0.9460	0.032*
Height (Z-score)	-0.2877	0.0570	0.187
BMI (Z-score)	-1.7445	-1.2060	0.217
Fat mass (kg)	8.203	8.360	0.922
Fat-free mass (kg)	10.123	20.200	0.268
Amenorrhea (months)	21.13	16.67	0.315

*significant at p<0.05; **significant at p<0.01; ***significant at p<0.001

Table VI – Bone metabolism parameters and mean comparison (t-test for independent samples) with presence of bone impairment (N-BMD *vs*. I-BMD) (n=51)

N-BMD: Normal bone mineral density

I-BMD: Impaired bone mineral density

Variables	Bone Impairment		p value
	Yes (I-BMD)	No (N-BMD)	
Calcium (mg/dL)	2.4100	2.4311	0.509
Phosphorus (mg/dL)	1.2247	1.2321	0.885
PTH (pg/mL)	33.608	30.492	0.367
BALP (U/L)	14.496	14.711	0.912
Vitamin D (ng/mL)	55.273	52.747	0.719
P1NP (mcg/L)	52.2935	77.5814	0.106

*significant at p<0.05; **significant at p<0.01; ***significant at p<0.001

Table VII – Clinical variables and mean comparison (one-way ANOVA) with degree of bone impairment (N-BMD vs. RL-BMD vs. L-BMD) (n=51)

N-BMD: Normal bone mineral density

RL-BMD: At risk for low bone mineral density

L-BMD: Low bone mineral density

Variables	Degree of bone impairment			p value
	N-BMD	RL-BMD	L-BMD	
Age (years)	15.2640	16.8767	17.2619	<0.001***
Pre-morbid age (years)	13.0950	14.5529	14.3679	0.003**
Duration of illness (years)	2.1690	2.4043	2.8421	0.260
Pre-morbid weight (Z-score)	0.2565	0.1457	-0.3943	0.037*
Current weight (Z-score)	-0.9460	-1.1600	-2.2350	0.018*
Height (Z-score)	0.0570	0.0227	-0.5787	0.072
BMI (Z-score)	-1.2060	-1.3407	-2.1231	0.164
Fat mass (kg)	8.360	9.387	7.094	0.521
Fat-free mass (kg)	20.200	20.140	18.169	0.141
Amenorrhea (months)	16.67	21.00	21.27	0.608

*significant at p<0.05; **significant at p<0.01; ***significant at p<0.001

Table VIII – Bone metabolism parameters and mean comparison (one-way ANOVA) with degree of bone impairment (N-BMD *vs*. RL-BMD *vs*. L-BMD) (n=51)

N-BMD: Normal bone mineral density

RL-BMD: At risk for low bone mineral density

L-BMD: Low bone mineral density

Variables	Degree of bone impairment			p value
	N-BMD	RL-BMD	L-BMD	
Calcium (mg/dL)	2.4311	2.4100	2.4100	0.806
Phosphorus (mg/dL)	1.2321	1.2078	1.2400	0.882
PTH (pg/mL)	30.492	33.943	33.217	0.676
BALP (U/L)	14.711	14.613	14.362	0.989
Vitamin D (ng/mL)	52.747	64.482	46.064	0.120
P1NP (mcg/L)	77.5814	47.7671	55.4620	0.260

*significant at p<0.05; **significant at p<0.01; ***significant at p<0.001

Table IX – Clinical variables and mean comparison (t-test for independent samples) within bone impairment group (RL-BMD vs. L-BMD) (n=31)

Variables	Groups with bone Impairment		p value
	At risk of low BMD (RL-BMD)	Low BMD (L-BMD)	
Age (years)	16.8767	17.2619	0.179
Pre-morbid age (years)	14.5529	14.3679	0.370
Duration of illness (years)	2.4043	2.8421	0.156
Pre-morbid weight (Z-score)	0.1457	-0.3943	0.019*
Current weight (Z-score)	-1.1600	-2.2350	0.017*
Height (Z-score)	0.0227	-0.5787	0.042*
BMI (Z-score)	-1.3407	-2.1231	0.075
Fat mass (kg)	9.387	7.094	0.113
Fat-free mass (kg)	20.140	18.169	0.022*
Amenorrhea (months)	21.00	21.27	0.475

*significant at p<0.05; **significant at p<0.01; ***significant at p<0.001

Table X – Bone metabolism parameters and mean comparison (t-test for independent samples) within bone impairment group (RL-BMD vs. L-BMD) (n=31)

Variables	With bone impairment		p value
	At risk of low BMD (RL-BMD)	Low BMD (L-BMD)	
Calcium (mg/dL)	2.4100	2.4100	0.500
Phosphorus (mg/dL)	1.2078	1.2400	0.283
PTH (pg/mL)	33.943	33.217	0.457
BALP (U/L)	14.613	14.362	0.455
Vitamin D (ng/mL)	64.482	46.064	0.050
P1NP (mcg/L)	47.7671	55.4620	0.297

*significant at p<0.05; **significant at p<0.01; ***significant at p<0.001

Table XI – Clinical variables and mean comparison (t-test for independent samples) between "normal and at risk for low BMD" with "low BMD" (N-BMD + RL-BMD *vs*. L-BMD) (n=51)

N-BMD: Normal bone mineral density

RL-BMD: At risk for low bone mineral density

L-BMD: Low bone mineral density

Variables	Bone Impairment		p value
	N-BMD + RL-BMD	L-BMD	
Age (years)	15.9551	17.2619	0.007**
Pre-morbid age (years)	13.6953	14.3679	0.072
Duration of illness (years)	2.2659	2.8421	0.061
Pre-morbid weight (Z-score)	0.2109	-0.3943	0.011*
Current weight (Z-score)	-1.0377	-2.2350	0.005**
Height (Z-score)	0.0423	0.5787	0.021*
BMI (Z-score)	-1.2637	-2.1231	0.029*
Fat mass (kg)	8.800	7.094	0.156
Fat-free mass (kg)	20.174	18.169	0.047*
Amenorrhea (months)	19.14	21.27	0.307

*significant at p<0.05; **significant at p<0.01; ***significant at p<0.001

Table XII – Bone metabolism parameters and mean comparison (t-test for independent samples) between “normal and at risk for low BMD” with “low BMD” (N-BMD + RL-BMD *vs*. L-BMD) (n=51)

N-BMD: Normal bone mineral density

RL-BMD: At risk for low bone mineral density

L-BMD: Low bone mineral density

Variables	Severity of bone disease		p value
	N-BMD + RL-BMD	L-BMD	
Calcium (mg/dL)	2.4215	2.4100	0.365
Phosphorus (mg/dL)	1.2226	1.2400	0.345
PTH (pg/mL)	31.763	33.217	0.363
BALP (U/L)	14.667	14.362	0.442
Vitamin D (ng/mL)	57.357	46.064	0.070
P1NP (mcg/L)	67.6433	55.4620	0.237

*significant at p<0.05; **significant at p<0.01; ***significant at p<0.001

BIBLIOGRAPHY

1. Feeding and Eating Disorders. In: *Diagnostic and Statistical Manual of Mental Disorders*. American Psychiatric Association Publishing; 2022. doi:10.1176/appi.books.9780890425787.x10_Feeding_and_Eating_Disorders
2. Hay P. Current approach to eating disorders: a clinical update. *Intern Med J*. 2020;50(1):24-29. doi:10.1111/imj.14691
3. Wszołek M, Ziora-Jakutowicz K, Gorczyca P, Rojewska K, Ziora K. Assessment of the frequency of hormonal disorders of the gonads and bone mineral density among women treated for anorexia nervosa in adolescence. *Pediatr Endocrinol Diabetes Metab*. 2022;28(1):16-22. doi:10.5114/pedm.2022.112866
4. Workman C, Blalock D V., Mehler PS. Bone density status in a large population of patients with anorexia nervosa. *Bone*. 2020;131. doi:10.1016/j.bone.2019.115161
5. Drabkin A, Rothman MS, Wassenaar E, Mascolo M, Mehler PS. Assessment and clinical management of bone disease in adults with eating disorders: A review. *J Eat Disord*. 2017;5(1). doi:10.1186/s40337-017-0172-0
6. Bemer P, Di Lodovico L, Haykanush O, et al. Bone mineral density at extremely low weight in patients with anorexia nervosa. *Clin Endocrinol (Oxf)*. 2021;95(3):423-429. doi:10.1111/cen.14498
7. Achamrah N, Coëffier M, Jésus P, et al. Bone Mineral Density after Weight Gain in 160 Patients with Anorexia Nervosa. *Front Nutr*. 2017;4. doi:10.3389/fnut.2017.00046
8. Jada K, Djossi SK, Khedr A, Neupane B, Proskuriakova E, Mostafa JA. The Pathophysiology of Anorexia Nervosa in Hypothalamic Endocrine Function and Bone Metabolism. *Cureus*. Published online December 21, 2021. doi:10.7759/cureus.20548
9. Mehler PS, Brown C. Anorexia nervosa - Medical complications. *J Eat Disord*. 2015;3(1). doi:10.1186/s40337-015-0040-8
10. Zadeh P, Etheridge L. Should teenage girls with anorexia nervosa be offered oestrogen treatment to reduce later risk of osteoporosis? *Arch Dis Child*. 2020;105(8):798-802. doi:10.1136/archdischild-2019-318571
11. Misra M, Klibanski A. Endocrine consequences of anorexia nervosa. *Lancet Diabetes Endocrinol*. 2014;2(7):581-592. doi:10.1016/S2213-8587(13)70180-3
12. Baxter-Jones ADG, Faulkner RA, Forwood MR, Mirwald RL, Bailey DA. Bone mineral accrual from 8 to 30 years of age: An estimation of peak bone mass. *Journal of Bone and Mineral Research*. 2011;26(8):1729-1739. doi:10.1002/jbmr.412

13. Dirckx N, Moorer MC, Clemens TL, Riddle RC. The role of osteoblasts in energy homeostasis. *Nat Rev Endocrinol*. 2019;15(11):651-665. doi:10.1038/s41574-019-0246-y
14. Søeby M, Gribsholt SB, Clausen L, Richelsen B. Fracture Risk in Patients with Anorexia Nervosa Over a 40-Year Period. *Journal of Bone and Mineral Research*. 2023;38(11):1586-1593. doi:10.1002/jbmr.4901
15. Högler W, Ward L. Osteoporosis in Children with Chronic Disease. *Endocr Dev*. 2015;28:176-195. doi:10.1159/000381045
16. El Ghoch M, Gatti D, Calugi S, Viapiana O, Bazzani PV, Grave RD. The association between weight gain/restoration and bone mineral density in adolescents with anorexia nervosa: A systematic review. *Nutrients*. 2016;8(12). doi:10.3390/nu8120769
17. Legroux I, Cortet B. Factors influencing bone loss in anorexia nervosa: Assessment and therapeutic options. *RMD Open*. 2019;5(2). doi:10.1136/rmdopen-2019-001009
18. Gielen E, Bergmann P, Bruyère O, et al. Osteoporosis in Frail Patients: A Consensus Paper of the Belgian Bone Club. *Calcif Tissue Int*. 2017;101(2):111-131. doi:10.1007/s00223-017-0266-3
19. Zipfel S, Giel KE, Bulik CM, Hay P, Schmidt U. Anorexia nervosa: Aetiology, assessment, and treatment. *Lancet Psychiatry*. 2015;2(12):1099-1111. doi:10.1016/S2215-0366(15)00356-9
20. Williams C, Sapra A. *Osteoporosis Markers*.; 2024.
21. *2019 ISCD Official Positions Pediatric*.; 2019.
22. Svedlund A, Pettersson C, Tubic B, et al. Bone mass and biomarkers in young women with anorexia nervosa: a prospective 3-year follow-up study. *J Bone Miner Metab*. 2022;40(6):974-989. doi:10.1007/s00774-022-01359-x
23. Winkler LAD, Frølich JS, Schulpen M, Støving RK. Body composition and menstrual status in adults with a history of anorexia nervosa—at what fat percentage is the menstrual cycle restored? *International Journal of Eating Disorders*. 2017;50(4):370-377. doi:10.1002/eat.22600
24. Moskowitz L, Weiselberg E. Anorexia Nervosa/Atypical Anorexia Nervosa. *Curr Probl Pediatr Adolesc Health Care*. 2017;47(4):70-84. doi:10.1016/j.cppeds.2017.02.003
25. Solmi M, Veronese N, Correll CU, et al. Bone mineral density, osteoporosis, and fractures among people with eating disorders: A systematic review and meta-analysis. *Acta Psychiatr Scand*. 2016;133(5):341-351. doi:10.1111/acps.12556
26. Schorr M, Klibanski A. Anorexia nervosa and bone. *Curr Opin Endocr Metab Res*. 2018;3:74-82. doi:10.1016/j.coemr.2018.01.002

27. Donaldson AA, Feldman HA, O'Donnell JM, Gopalakrishnan G, Gordon CM. Spinal Bone Texture Assessed by Trabecular Bone Score in Adolescent Girls With Anorexia Nervosa. *J Clin Endocrinol Metab*. 2015;100(9):3436-3442.
28. Robinson L, Aldridge V, Clark EM, Misra M, Micali N. A systematic review and meta-analysis of the association between eating disorders and bone density. *Osteoporosis International*. 2016;27(6):1953-1966. doi:10.1007/s00198-015-3468-4
29. Jagielska G, Przedlacki J, Bartoszewicz Z, Racicka E. Zaburzenia mineralizacji kości jako powikłanie jadłowstrętu psychicznego - etiologia, rozpowszechnienie, przebieg i leczenie. *Psychiatr Pol*. 2016;50(3):509-520. doi:10.12740/PP/59289
30. Ostrowska Z, Morawiecka-Pietrzak M, Pluskiewicz W, et al. The relationship between chemerin, bone metabolism, the RANKL/RANK/OPG system, and bone mineral density in girls with anorexia nervosa. *Endokrynol Pol*. 2022;73(1):26-34. doi:10.5603/EP.a2021.0103
31. Motyl KJ, Guntur AR, Carvalho AL, Rosen CJ. Energy Metabolism of Bone. *Toxicol Pathol*. 2017;45(7):887-893. doi:10.1177/0192623317737065
32. Lu Y, Lu P, Lin L, Chen H, Zhang F, Li X. Characteristics of bone mineral density in patients with functional hypothalamic amenorrhoea and its association with reproductive hormones and body composition. *Clin Endocrinol (Oxf)*. 2024;100(4):358-365. doi:10.1111/cen.15016
33. Indirli R, Lanzi V, Mantovani G, Arosio M, Ferrante E. Bone health in functional hypothalamic amenorrhea: What the endocrinologist needs to know. *Front Endocrinol (Lausanne)*. 2022;13. doi:10.3389/fendo.2022.946695

INSTITUTO DE **CIÊNCIAS BIOMÉDICAS ABEL SALAZAR**