Maria Manuela Lobato Guimarães Ferreira Cabral

Vitamin D and metabolic syndrome among Portuguese adolescents: The EPITeen cohort

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Orientador: Professora Doutora Elisabete Ramos,
Professora Auxiliar na Faculdade de Medicina da Universidade do Porto

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Esta dissertação teve por base um manuscrito, apresentado na secção de resultados. Para a sua elaboração fui responsável pela análise dos dados e pela redação da versão inicial.

- Vitamin D intake and metabolic syndrome among 13-year-old Portuguese adolescents.

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Table of Contents

RESUMO

Introdução: Durante décadas, a vitamina D foi estudada sob o prisma da patologia óssea. Porém, estudos recentes têm sugerido uma possível associação entre valores baixos desta vitamina e aumento de risco de doença cardiovascular. Uma vez que, o síndrome metabólico e seus componentes começam frequentemente a ser estabelecidos na adolescência, aumentar o conhecimento sobre esta etapa de vida é necessário, a fim de identificar as estratégias para prevenir doença e morbilidade na vida adulta.

Objectivo: Este trabalho teve como objetivo avaliar a associação entre a ingestão de vitamina D com o síndrome metabólico e seus componentes em adolescentes de 13 anos.

Métodos: Foi realizada uma análise transversal de 1.033 adolescentes de 13 anos, membros da coorte "*Epidemiological Health Investigation of Teenagers in Porto*" (EPITeen) que nasceram em 1990 e estavam matriculados em escolas públicas e privadas da cidade do Porto, durante o ano letivo de 2003/2004. Através de dois questionários estruturados (um respondido em casa, o outro pelos adolescentes na escola), foram recolhidos dados sociodemográficos, comportamentais e sobre história individual e familiar de doença. A ingestão de vitamina D foi avaliada através de um questionário de frequência alimentar. Uma equipa de profissionais de saúde realizou medições antropométricas, aferiu a pressão arterial e colheu uma amostra de sangue em jejum. Os componentes do síndrome metabólico foram definidos de acordo com a definição modificada para a idade do "National Cholesterol Education Program Adult Treatment Panel III". A associação entre a ingestão de vitamina D e os componentes do síndrome metabólico foi calculada através de modelos de regressão logística.

Resultados: A mediana de ingestão de vitamina D (P25-P75) foi 4,11 (2,88-5,60) µg; sendo superior nos adolescentes que reportaram a toma de suplementos vitamínicos e inferior nos adolescentes classificados como obesos ou com obesidade abdominal. A prevalência de síndrome metabólico foi de 13,2%. Com a diminuição da ingestão de vitamina D, o *odds ratio* (intervalo de confiança de 95%) para colesterol HDL baixo aumentou até 1,22 (0,84-1,78) e até 1,78 (1,00-3,17) para IMC elevado.

Conclusões: Apesar da extremamente baixa ingestão de vitamina D, foi observada uma relação inversa com o IMC elevado e com colesterol HDL baixo, embora sem significado estatístico. Nenhuma tendência foi encontrada para o *odds* de ter síndrome metabólico.

Palavras-chave: vitamina D, síndrome metabólica, adolescentes.

ABSTRACT

Introduction: For decades, a great deal of attention towards vitamin D deficiency has been justifiably placed on understanding the consequences of its role on bone health. However, growing evidence suggests a possible association between low vitamin D levels and increased cardiovascular risk. Since the metabolic syndrome and its components often begin to be established throughout adolescence, research regarding this stage of life is needed, in order to prevent illness and morbidity in adulthood.

Objective: This research aimed to evaluate the association of vitamin D intake with metabolic syndrome and its components in 13-year-old adolescents.

Methods: We conducted a cross-sectional analysis of 1033 adolescents aged 13-years-old, members of the Epidemiological Health Investigation of Teenagers in Porto (EPITeen), who were born in 1990 and were enrolled at public and private schools in Porto, during the 2003/2004 school year. Data on behavioral, social and demographic characteristics and individual and family history of disease were collected using two standardized questionnaires (one fulfilled at home and the other by the adolescents at school). Vitamin D intake was assed by a food frequency questionnaire. A team of experienced nurses, nutritionists and physicians performed anthropometric assessment, blood pressure measurement and fasting blood sample collection. Metabolic syndrome components were defined according to the National Cholesterol Education Program Adult Treatment Panel III definition modified for age. Logistic regression was performed in order to estimate the association between vitamin D intake and metabolic syndrome components.

Results: Median (P25-P75) vitamin D intake was 4.11 (2.88-5.60) μg; it was higher among adolescents who reported the use of vitamin supplements and lower in those classified as obese or abdominally obese. Metabolic syndrome prevalence was 13.2%. The adjusted odds ratio (95% confidence interval) increased with the decrease of vitamin D intake until 1.22 (0.84-1.78) for low HDL cholesterol and 1.78 (1.00-3.17) for high BMI.

Conclusions: Notwithstanding the extremely low vitamin D intake, an inverse relation with high BMI and low HDL cholesterol was observed, though without statistical significance. No trend was found for metabolic syndrome.

Key words: Vitamin D, metabolic syndrome, adolescents.

LIST OF TABLES

LIST OF ABBREVIATIONS

1,25-dihydroxyvitamin D= calcitriol

25OHD= calcidiol

ACE= angiotensinogen-converting enzyme

AHA/NHLBI= American Heart Association/National Heart, Lung, and Blood Institute

Ang= angiotensin

BMI= body mass index

CDC: Center for Disease Control and Prevention

CHD= coronary heart disease

CI: confidence interval

CVDs= Cardiovascular diseases

DALYs= disability-adjusted life years

DPB= specific carrier vitamin D-binding

EPITeen= Epidemiological Health Investigation of Teenagers in Porto

FFQ: Food Frequency Questionnaire

FGF23= fibroblast-like growth factor-23

HDL cholesterol= high-density lipoprotein cholesterol

IDF= International Diabetes Federation

IL= interleukin

IOM= Institute of Medicine

Kg/m^2: kilograms per meter square

LDL cholesterol= low-density lipoprotein cholesterol

MetS= metabolic syndrome

NHANES= National Health and Nutrition Examination Survey

OR= odds ratio

PDAY study= Pathobiological determinants of atherosclerosis in youth

PAP study= Prevalence, awareness, treatment and control of hypertension in Portugal

PTH= parathyroid hormone

RAS= renin-angiotensin system

SD= Standard Deviation

SPSS= Statistical Package for the Social Sciences

VDREs= vitamin D - responsive elements

INTRODUCTION

1. The burden of cardiovascular disease

Of the estimated 57 million global deaths in 2008, 36 million (63%) were due to noncommunicable diseases[1]. Population growth and increased longevity are leading to a rapid increase in the total number of middle-aged and older adults, with a corresponding increase in the number of deaths caused by noncommunicable diseases. Projections are that the total number of annual noncommunicable disease deaths will reach 55 million by 2030, whereas infectious disease deaths are projected to decline over the next 20 years[1].

Cardiovascular diseases (CVDs), which include diseases of the heart, brain vasculature and blood vessels, remain the leading cause of death and disability worldwide.[2] Even though a large proportion of CVDs is preventable, they continue to rise mainly because preventive measures are inadequate[3]. Over the past two decades, cardiovascular mortality rates have declined substantially in high-income countries due to a combination of prevention and control measures, which provide evidence that investment in prevention is the most sustainable solution for the CVD epidemic[4, 5]. Even so, it is projected that the annual number of deaths due to CVD will increase to 25 million in 2030[1].

CVDs are also the main cause of death in Europe accounting for 4 million deaths each year, with coronary heart disease (CHD) being the single most common cause of death in Europe accounting for 1.8 million deaths each year[6]. Stroke by itself is the second single most common cause of death in Europe accounting for almost 1.1 million deaths each year. Just under half of all deaths from CVD in both men and women are from CHD, with stroke accounting for nearly a third of deaths in women and a quarter of deaths in men[6].

In Portugal, CVDs are the main cause of death, increasing from 26.4% in 1960 to 38.7% in 2000. Portugal has the highest stroke mortality rate in Western Europe, and although reducing dramatically over the past two decades, it remains higher than that for most European countries. In contrast, coronary heart disease mortality is low and similar to Spain and Italy, countries with historically low coronary heart disease rates too [6].

One of the main underlying pathological processes that leads to heart attacks (coronary heart disease) and strokes (cerebrovascular disease) is atherosclerosis.[2] The early changes of atherosclerosis develop in childhood and adolescence due to the overall effect of a number of risk factors [5, 7-10].

Atherosclerosis involves an inflammatory process affecting medium and large-sized blood vessels throughout the cardiovascular system[11, 12]. When the lining (endothelium) of these blood vessels is exposed to raised levels of low-density lipoprotein cholesterol (LDL cholesterol) and certain other substances, such as free radicals, the endothelium becomes permeable to lymphocytes and monocytes. These cells migrate into the deep layers of the wall of the blood vessel. A series of reactions occur, attracting LDL cholesterol particles to the site. These particles are engulfed by monocytes, which are then transformed into macrophages (foam cells). Smooth muscle cells migrate to the site from deeper layers of the vessel wall (the media). Later, a fibrous cap consisting of smooth muscle and collagen is formed. At the same time, the macrophages involved in the original reaction begin to die, resulting in the formation of a necrotic core covered by the fibrous cap. These lesions (atheromatous plaques) enlarge as cells and lipids accumulate in them and the plaque begins to bulge into the vessel lumen. As the process continues, there is thinning of the fibrous cap accompanied by fissuring of the endothelial surface of the plaque, which may rupture. With the rupture of the plaque, lipid fragments and cellular debris are released into the vessel lumen. These are exposed to thrombogenic agents on the endothelial surface, resulting in the formation of a thrombus. If the thrombus is large enough, and a coronary blood vessel or a cerebral blood vessel is blocked, this results in a heart attack or stroke[11, 12]. This process can develop over many years, starting in the childhood and manifesting as heart attacks and strokes in people of middle age[2].

Unhealthy behaviours, lead to metabolic/physiological changes: raised blood pressure (hypertension); overweight/obesity; raised blood sugar (diabetes); and raised blood lipids (dyslipidaemia), causing damage to coronary and cerebral blood vessels due to the aforementioned atherosclerotic process.

These behavioural and metabolic risk factors often coexist in the same person and act synergistically to increase the individual's risk of developing acute vascular events such as heart attacks and strokes[2]. The clustering of these risk factors, which is associated to insulin resistance and co-occur more often than it might be expected by chance, is called the *Metabolic Syndrome* (MetS)[13]. Strong scientific evidence demonstrates that reducing total cardiovascular risk results in the prevention of heart attacks and strokes, being proven its cost-effectiveness[9].

Given its role throughout the life course in the promotion and maintenance of good health, it is important to highlight the considerable body of evidence regarding the nutritional background of CVDs, since food intake is a relevant modifiable

determinant, which, indeed, can contribute to a healthy body weight, an appropriate lipid profile and a desirable blood pressure[14].

1.1. Risk factors begin at early stages

Low birth weight is associated to an increased risk of adult diabetes and CVD. There is increasing evidence that exposure to under nutrition in early life increases an individual's vulnerability to these disorders by "programming" permanent metabolic changes[15-17]. Behavioural risk factors such as tobacco use or dietary habits are learned in childhood/adolescence and continue into adulthood.

Additionally, in many countries, metabolic risk factors such as obesity and diabetes are starting to appear at early ages[9].

The importance of the early identification of children at risk of developing the MetS and subsequently progressing to type 2 diabetes and cardiovascular disease in later life is critical[18, 19]. From birth and before, circumstances can predispose a child to conditions such as obesity or dysglycaemia. The presence of maternal gestational diabetes[20], low birth weight[21], infant feeding practices[22], early adiposity rebound[23], and genetic factors may all contribute to a child's future level of risk. Likewise, being raised in an "obesogenic" environment can also have a strong impact, as can the influence of socioeconomic factors with weight gain often being observed as a positive correlate to affluence in developing countries [24].

Cluster-tracking studies have shown that multiple cardiovascular risk factors persist from childhood into adulthood in 25-60% of cases[25, 26]. Changes of atherosclerosis within blood vessels can begin in the first decade of life as fatty streaks and plaques[9, 27]. However, this may be modifiable as shown by Raitakari *et al.,* who disclosed that subjects who either developed or lost their risk factor clustering over time had significant changes in their adiposity and lifestyle behaviours related to nutrition and physical activity[28].

Life course epidemiology was built on the premise that various biological and social factors throughout life independently, cumulatively and interactively influence health and disease in adult life[29]. Adverse health outcomes in adulthood can frequently be traced back to disadvantaged conditions earlier on. Such conditions may accompany birth and the early years of life, but they may also arise from later experiences and personal choices. Therefore, it is of utmost importance to adopt

measures that promote population's health at an early stage, preventing illness and morbidity later in life.

1.2. Metabolic disorders

1.2.1. Overweight and Obesity

To achieve optimal health, the median body mass index (BMI), a measure of weight relative to height, for adult populations should be in the range of 21–23 kg/m^2, while the goal for individuals should be to maintain a BMI in the range 18.5–24.9 kg/m^2[9].

Nevertheless, obesity is a growing health problem in both developed and developing countries. Worldwide, at least 2.8 million people die each year as a result of being overweight or obese, and an estimated 35.8 million (2.3%) of global disability-adjusted life years (DALYs) are caused by overweight or obesity[30]. In 2008, 34.0% of adults over the age of 20 were overweight with a BMI greater than or equal to 25 kg/m^2 (33.6% of men and 35.0% of women). Regarding obesity (BMI greater than or equal to 30 kg/m^2) 9.8% of men and 13.8% of women were obese compared to 4.8% for men and 7.9% for women in 1980[30].

Prospective epidemiological studies have shown a relationship between overweight or obesity and total mortality, aside from cardiovascular morbidity and mortality. In fact, risks of coronary heart disease, ischemic stroke and type 2 diabetes mellitus increase steadily with an increasing BMI[31]. Obesity is strongly related to major cardiovascular risk factors such as raised blood pressure, glucose intolerance, type 2 diabetes and dyslipidaemia[9, 30].

BMI is commonly used to classify obesity among adults and is recommended for use with children. Cut-off criteria are based on the Center for Disease Control and Prevention's 2000 BMI-for-age growth charts for the United States[32]. Based on current recommendations of expert committees, children and adolescents with BMI values at or above the 95th percentile of the sex- specific BMI growth charts are categorized as obese[33]. Regardless of the classification used, overweight has become increasingly prevalent in many countries, especially in those economically developed, not only in adult population, but also in children and adolescents.

Childhood and adolescence are critical periods for the onset of obesity[34, 35]. Despite being usually considered as an adult problem, it is increasing in children and

adolescents worldwide, already showing some of the aforementioned health complications that track into adulthood and may lead to major disorders[6, 31].

Results from the 2009–2010 United States National Health and Nutritional Examination Survey (NHANES), estimated that 16.9% of children and adolescents between 2–19 years old are obese. Between 1976–1980 and 2009–2010, the prevalence of obesity increased. However, between 1999–2000 and 2009–2010, no significant trend was observed in obesity prevalence in girls, although a significant increase was seen in boys. Despite the observation that the trend seems to be levelling off, it remains worrisome the high prevalence of obesity observed among youth[33].

Also in Portugal, a recent systematic review verified that in the period comprised between 1995 and 2005, the mean BMI and the prevalence of overweight/obesity had a marked increase among younger subjects (20-years-old), which allow to recognize that a future increase in the overall burden of obesity in the younger Portuguese generation can be expected[36].

1.2.2. Hypertension

Worldwide, high blood pressure is estimated to cause 7.5 million deaths, about 12.8% of the total of all annual deaths[37]. Globally, the overall prevalence of high blood pressure in adults aged 25 and over was around 40% in 2008. The number of people with uncontrolled hypertension has risen from 600 million in 1980 to nearly one billion in 2008[30].

Brent *et al* observed the US trends in prevalence, awareness, treatment, and control of hypertension between 1988 and 2008 and verified that rates of hypertension increased from 23.9% in 1988-1994 to 28.5% in 1999-2000, but did not change between 1999-2000 and 2007-2008. They concluded that hypertension control improved, with most of the progress toward the Healthy People 2010 goal of controlling BP in 50% of all individuals with hypertension occurring between 1999 and 2008. However, prevalent hypertension is not decreasing toward the national goal of 16% and will likely remain high unless adverse trends in population nutrition and BMI occur or pharmacological approaches to hypertension prevention are adopted[38].

In Portugal, the estimated prevalence of hypertension is around 40%[39], being the most common self-reported chronic disease in the National Health Survey conducted in 2005/2006, with a prevalence of 19.8% and with a frequency higher

than 80% in the age groups above the 45-54 years[40]. These data that is consistent with the PAP study (Prevalence, awareness, treatment and control of hypertension in Portugal), where it was found that only 45.7% of participants with hypertension were diagnosed, of whom 38.9% were treated and among these only 28.8% had values below 140/80 mmHg for systolic and / or diastolic. The younger age groups were those who had a lower rate of diagnosis and treatment[41].

Long-term follow-up studies have shown that blood pressure levels measured in childhood and adolescence are positively associated with blood pressure in early adulthood, and that there is a clear association between elevated blood pressure level in late adolescence or young adulthood and cardiovascular morbidity and mortality[42].

In a cross-sectional study, Ramos *et al.* evaluated 2023 Portuguese adolescents and observed a prevalence of hypertension of 22.0% and 13.3% for prehypertension. The prevalence of hypertension was higher in males (25.4% vs. 18.8%), but for both genders blood pressure increased with body mass index. [43]

Early detection of hypertension and treatment to reduce cardiovascular risk in people with hypertension is vital for prevention of stroke and heart attacks. Undetected and uncontrolled hypertension increasing the cardiovascular risk is a major contributor to stroke worldwide. The stroke and heart attack risk of people with high cardiovascular risk and/or raised blood pressure can be reduced through non-pharmacological (e.g. low salt diet, physical activity) and pharmacological measures[30]. Multicountry studies have shown large differences in mean population blood pressure, associated with variations in adiposity and dietary salt[44].

The trend in recent decades for higher blood pressure among children and adolescents[45] means that hypertension will remain a major public health concern.

1.2.3. Impaired glucose

Diabetes is a major risk factor for CVD. In 2008, diabetes was responsible for 1.3 million deaths globally. The global prevalence of diabetes was estimated to be 10%[30]. Lack of early detection and care for diabetes results in severe complications, including heart attacks, strokes, renal failure, amputations and blindness[30].The risk of cardiovascular events is from two to three times higher in people with type 1 or type 2 diabetes and the risk is disproportionately higher in women[46, 47].

Both impaired glucose tolerance and impaired fasting glycaemia are risk categories for future development of diabetes and CVD[9]. Studies indicate that the

risk of cardiovascular morbidity and mortality is already significantly increased in those with modestly elevated impaired fasting glucose levels, even if they are below the cut-off for diabetes[48, 49].

Furthermore, abnormal glucose regulation tends to occur together with other known cardiovascular risk factors such as central obesity, elevated blood pressure, low HDL cholesterol and a high triglyceride level[50, 51].

Despite the low prevalence in children and adolescents[52], the increase in BMI has also increased the prevalence of this risk factor in this age group. Once they are usually considered as being healthy, many of them seek health care infrequently, only for acute problems, with the result that they may go years without contact with a health care provider.

1.2.4. Dyslipidaemia

Raised blood cholesterol increases the risk of heart disease and stroke. Globally, one third of ischemic heart disease is attributable to high cholesterol[9]. Overall, raised cholesterol is estimated to cause 2.6 million deaths (4.5% of total) and 29.7 million DALYS, or 2% of total DALYS globally. In 2008, the prevalence of raised total cholesterol among adults, defined as total cholesterol ≥ 6.2 mmol/l (240 mg/dl), was 9.7% (8.5% for males and 10.7% for females)[30].

Lowering raised serum cholesterol reduces the risk of heart disease. For example, a 10% reduction in serum cholesterol in 40-year old men has been reported to result in a 50% reduction in heart disease within five years; the same serum cholesterol reduction for 70-year old men can result in an average 20% reduction in heart disease occurrence within five years[30].

However, it is important to consider that the lipoprotein profile includes: low-density lipoprotein cholesterol (LDL), high-density lipoprotein cholesterol (HDL) and triglycerides, each component having a different role in the development of CVD. LDL cholesterol and triglycerides are central for the development of CVD, namely by their role on the development of atherosclerosis, contrasting with HDL cholesterol that protects against vascular disease [2]. Thus, the lipid profile with elevated LDL cholesterol and triglycerides, and low HDL cholesterol, conditions an increased risk of cardiovascular disease.

The PDAY study (Pathobiological determinants of atherosclerosis in youth) and the Bogalusa Heart Study were the first studies to document anatomic changes and to relate them with dyslipidaemia. In the PDAY study, arteries and tissues from

more than 2000 persons 15-34 years of age, whose deaths were attributed to accidents, homicides or suicides, were collected and lipids at time of autopsy were analysed. They found that the prevalence of increased coverage of the arterial intimal surface, with fatty streaks and fibrous plaques, was associated with elevation of serum cholesterol levels[53]. The Bogalusa investigators followed a total and geographically well-defined community in Bogalusa, USA. In the autopsy studies done to persons who had died for accidental causes, they also found significant association between atherosclerotic lesions and abnormalities of all lipid components[54].

In a Portuguese study, which aimed at access prevalence of dyslipidaemia and to identify associated characteristics in 1388 13-year-old participants, dyslipidaemia was highly prevalent, with presence in 24.5% of the adolescents, similar in boys and girls, with family history of dyslipidaemia and BMI being the main determinants of dyslipidaemia at this age[55].

Given that dyslipidaemia is a major contributing factor for atherosclerosis and consequently for cardiovascular diseases, and once its process begins in the first decades of life[56], the importance of primary prevention in children and adolescents must be reinforced.

1.3. The metabolic syndrome

The *metabolic syndrome* (MetS) is defined as a complex of interrelated risk factors for CVD and diabetes, characterized by a pro-inflammatory and pro-thrombotic state, which include elevated fasting glucose, raised blood pressure, elevated triglyceride levels, low high-density lipoprotein cholesterol levels, and obesity (particularly central adiposity)[13].

Various diagnostic criteria have been proposed by different organizations over the past decade. More recently, these have come from the *International Diabetes Federation* (IDF)[57] and the *American Heart Association/National Heart, Lung, and Blood Institute* (AHA/NHLBI)[58]. Their main difference lies in the measure for central obesity, with this being a compulsory component in the IDF definition, lower than in the AHA/NHLBI criteria, and ethnic specific[59].

It has been found that the syndrome has a rising prevalence worldwide, which relates largely to increasing obesity and sedentary lifestyles, being estimated that one quarter of the world's population has already MetS[60]. Due to the increasing rates of adult obesity and its association with insulin resistance and type 2 diabetes,

the National Cholesterol Education Program (NCEP) panel stated that the metabolic syndrome will soon have a greater impact on premature coronary artery disease than does tobacco[61].

This condition is appearing with increasing frequency in children and adolescents, driven by the growing obesity epidemic in this young population[62]. As childhood overweight increases, its medical complications are becoming more common and more frequently recognized[63]. For example, the prevalence of type 2 diabetes has risen dramatically among adolescents in the past 20 years[64]. Studies suggest that a substantial percentage of overweight children and adolescents may be afflicted with one or more of the components of the MetS[65, 66]. Besides, autopsy studies have revealed that overweight in adolescence is associated to accelerated coronary atherosclerosis, which makes recent trends even more worrying[67].

Even though MetS is associated to more than two-fold increase in the risk of cardiovascular events, there is a debate whether it adds information to that provided by its individual components or it can be regarded as an alternative risk prediction tool. In fact, it remains controversial whether the risk associated with metabolic syndrome is greater than the sum of the risk resulting from its component features. Definition disparity adds to this controversy[68].

Despite the disagreement over the terminology and diagnostic criteria related to the metabolic syndrome[69], there appears to be a consensus in the medical field that the term *metabolic syndrome* is acceptable to identify individuals at high risk of both type 2 diabetes and cardiovascular disease (CVD)[59].

This problem seems to be greater in children and adolescents. While one definition, although with gender and ethnicity specific cut-off points, is suitable for use in the at-risk adult population, transposing a single definition to children and adolescents is problematic, given the difficulty to choose the parameters needed for its diagnosis and the choice of adequate cut-offs. Moreover, puberty occurs at different chronological ages, therefore, for the same age, similar values of these factors may mean different disease risk. Blood pressure, lipid levels, and anthropometric variables change with age and pubertal development. Puberty impacts on fat distribution and is known to cause a decrease both in insulin sensitivity, of approximately 30% with a complementary increase in insulin secretion[70], and in adiponectin levels[71]. Therefore, single cut-off points cannot be used to define abnormalities in children. Instead, values above the 90th, 95th, or 97th percentile for gender and age are used.

The wide variety of cut-off points used has emphasize the need for a single consistent set of criteria, which can be easily measurable and used as the basis for

future work. Recently the IDF has proposed a new definition of the MetS for children and adolescents, with central obesity (waist circumference ≥ 90th percentile) being a "sine qua non" plus two or more of the following: systolic blood pressure ≥ 130 or diastolic blood pressure ≥ 85 mmHg, triglycerides ≥ 150mg/dl, HDL cholesterol ≤ 40 mg / dl and glucose ≥ 100mg/dl[72]. Inspired, in part, by the IDF definition in adults, this new definition builds on previous studies investigating the prevalence of MetS in children and adolescents, which have used modified adult criteria with varying cut-off points[73, 74] (table 1).

Table 1. A range of some published metabolic syndrome definitions in pediatrics.

	Cook et al. [73]	**de Ferranti et al. [74]**	**IDF [72]**
	Three or more of the following:	Three or more of the following:	Central obesity + ≥ 2 of 4 criteria
WC	≥ 90th percentile (age and sex specific, NHANES III)	≥ 75th percentile	≥ 90th percentile
Fasting glucose	≥ 110 mg/dL	≥ 110 mg/dL	≥ 100 mg/dL
Triglycerides	≥ 110 mg/dL (age specific, NCEP)	≥ 100 mg/dL	≥ 150 mg/dL
HDL-C	≤ 40 mg/dL (all ages/sexes, NCEP)	≤ 50mg/dL(boys aged 15-19 years ≤ 45mg/dl)	< 40mg/dL
Blood Pressure	≥ 90th percentile (age, sex and height specific, NHBPEP)	≥ 90th percentile	Systolic BP ≥ 130 or diastolic BP ≥ 85 mmHg

2. Vitamin D

2.1. Overview and Metabolism

Vitamin D has been discovered as the fourth vitamin (therefore the denomination). Its characterization and synthesis using photochemistry were achieved by Windaus in Göttingen, Germany, in 1923, who was awarded thereafter with the Nobel Prize in Chemistry[75]. Recognition of the complex activities of vitamin D on the calcium homeostasis and bone metabolism has been ignited due to its association with rickets in children and osteomalacia in adults[76].

For a long time, a great deal of attention has been justifiably placed on understanding the consequences of vitamin D deficiency on bone health[77-79]. However, for nearly three decades, studies on vitamin D broadened their horizon, knowing now that its functions go far beyond the regulation of bone metabolism[80-83].

Vitamin D, also known as calciferol, comprises a group of fat-soluble seco-sterols. The major forms are vitamin D_2 and vitamin D_3. Vitamin D_2 (ergocalciferol) is found in plants as the product of ultraviolet B irradiation of ergosterol. Vitamin D_3 (cholecalciferol) has its origin from 7-dehydrocholesterol and is the product of ultraviolet B irradiation of this compound after transiently passing through pre-vitamin D_3. The vitamin D_3 can either be synthesized in the human epidermis or consumed in the diet via the intake of animal-based foods. Both vitamin D_3 and vitamin D_2 are synthesized commercially and found in dietary supplements or fortified foods. The D_2 and D_3 forms differ only in their side chain structure. The differences do not affect metabolism (i.e. activation), and both forms function as pro-hormones [23]. In the following vitamin D will be referred to both Vitamin D_2 and D_3.

The classical actions of vitamin D, which by itself is inactive, are due to the functions of the active metabolite, calcitriol. 25OHD (calcidiol), the precursor of calcitriol, is the major circulating form of vitamin D. It circulates bound to a specific plasma carrier protein, vitamin D binding protein (DBP), which also transports vitamin D and calcitriol[84].

Vitamin D is considered biologically inactive until it undergoes two enzymatic hydroxylation reactions. The first takes place in the liver, mediated by the 25-hydroxylase (most likely cytochrome P450 2R1 [CYP2R1]), which forms 25-hydroxyvitamin D (25OHD). The second reaction takes place in the kidney, mediated by 1α-hydroxylase (CYP27B1), which converts 25OHD to the biologically active hormone, calcitriol (1,25-dihydroxyvitamin D). The 1α-hydroxylase gene is also

expressed in several extra-renal tissues, but its contribution to calcitriol formation in these tissues is unknown (table 2).

Two counter-acting hormones tightly regulate the renal synthesis of calcitriol, with up-regulation via parathyroid hormone (PTH) and down-regulation via fibroblast-like growth factor-23 (FGF23). Low serum phosphorus levels stimulate calcitriol synthesis, whereas high serum phosphorus levels inhibit it. Following its synthesis in the kidney, calcitriol binds to DBP in order to be transported to target organs. The biological actions of calcitriol, involve regulation of gene expression at the transcriptional level, and are mediated through binding to a vitamin D receptor (VDR), located primarily in the nuclei of target cells. Additional hydroxylation reactions, such as that mediated by CYP24A1, result in more polar metabolites with very limited or no apparent biological activity[85].The actions of calcitriol include the regulation of serum calcium and phosphate homeostasis and, in turn, the development and maintenance of bone health[85].

Non-classical functions are less well elucidated. After 1,25(OH)D enters the cell, it is transported through the micro-tubular network to the nucleus. After it enters the nucleus bound to its vitamin D receptor (VDR), it complexes with the retinoic acid X receptor to form a heterodimeric complex that seeks out specific DNA sequences known as vitamin D-responsive elements (VDREs). Once the 1,25(OH)2D3-VDR-retinoic acid X receptor complex binds to the VDRE, resulting in expression of the vitamin D-responsive gene[86]. VDRs are found fairly ubiquitously throughout the body in tissues not involved with calcium and phosphate homeostasis, and the presence of VDRs in these tissues implies that calcitriol may play a more general role or that ligands other than calcitriol can activate the VDR[84]. Furthermore, VDREs, considered the hallmark of vitamin D action, are present in a large number of human genes involved in a wide range of classical and non-classical roles, such as the regulation of cell proliferation, cell differentiation, and apoptosis. It has been suggested that calcitriol exerts immunomodulatory and anti-proliferative effects through autocrine and paracrine pathways. These wide-ranging actions of calcitriol have further been hypothesized to play a potential role in preventive or therapeutic action in cancer and chronic conditions such as autoimmune conditions, infections and cardiovascular disease[87-89].

Table 2. Terms used in reference to Vitamin D[90].

Terms:
Vitamin D—also referred to as *calciferol*
Vitamin D2—also referred to as *ergocalciferol*
Vitamin D3—also referred to as *cholecalciferol*
25OHD—25-hydroxyvitamin D also referred to as *calcidiol* or *calcifediol*; indicates no distinction between D_2 and D_3 forms.
Calcitriol—1,25-dihydroxyvitamin D_3 (Note: *Ercalcitriol* - refers to 1,25-dihydroxyvitamin D_2, but in this report, the term "calcitriol" will be used for both)
24,25(OH)2D—24,25-dihydroxyvitamin D

Due to its fat-soluble nature, dietary vitamin D (either D_2 or D_3) is incorporated into micelles with other lipids and absorbed into the intestine by passive diffusion. The efficient absorption of vitamin D is dependent upon the presence of fat in the lumen, which triggers the release of bile acids and pancreatic lipase. In turn, bile acids initiate the emulsification of lipids, pancreatic lipase hydrolyzes the triglycerides into monoglycerides and free fatty acids, and bile acids support the formation of lipid-containing micelles, which diffuse into enterocytes. Inside the absorptive cells, the vitamin is incorporated into chylomicrons, and enters the lymphatic system and finally the plasma, where it is delivered to the liver by chylomicron remnants or to specific carrier vitamin D-binding (DBP), or transcalciferin. The efficiency of this absorption process is approximately 50%. Vitamin D synthesized in the skin from cholesterol enters the capillary system and is transported by DBP and delivered to the peripheral tissues. Little Vitamin D is stored in the liver[91, 92].

Regardless of its origin, vitamin D is an inactive pro-hormone and must first be metabolized to its hormonal form before it can function. Once vitamin D enters the circulation from the skin or from the lymph, it is cleared by the liver or storage tissues within a few hours[93].

Vitamin D is converted in the liver to 25-hydroxyvitamin D [25(OH)D], the major circulating metabolite of vitamin D[85]. At this point, 25OHD bound to DBP circulates in the blood stream and, when calcitriol is required due to a lack of calcium (or lack of phosphate), 25OHD is 1α-hydroxylated in the kidney to form calcitriol, the active form, by the 1α-hydroxylase enzyme. This metabolic step is very tightly

regulated by blood calcium and phosphate levels through PTH and the phosphaturic hormone, FGF23, and constitutes the basis of the vitamin D endocrine system that is central to maintaining calcium and phosphate homeostasis[85]. FGF23 acts by reducing the expression of renal sodium–phosphate transporters and reducing serum calcitriol levels. All naturally occurring vitamin D compounds interact with DBP. Calcitriol and vitamin D have significantly lower affinity for this protein than does 25OHD. Whereas vitamin D has an average lifetime in the body of approximately 2 months, 25OHD has a lifetime of 15 days, and calcitriol has a lifetime measured in hours[94]. Because of this relative stability, the principal biomarker used to assess vitamin D status is plasma 25-hydroxyvitamin D concentration. Aside from these key elements in vitamin D metabolism, more than 30 other metabolites have been found, but their importance seems minimal[94].

Adipose tissue stores of vitamin D probably represent “non-specific” stores sequestered because of the hydrophobic nature of vitamin D, but the extent to which the processes of accumulation or mobilization are regulated by normal physiological mechanisms remains unknown. Since vitamin D is stored in body fat and unavailable for use, body fat content is considered a factor that decreases its bioavailability. Studies show that body mass index and body fat content have an inverse relation to serum levels of 25 hydroxyvitamin D[95, 96].

The products of vitamin D metabolism are excreted through the bile into the feces, and very little is eliminated through the urine. This is in part due to renal reuptake of vitamin D metabolites bound to DBP, as mediated by the cubilin–megalin receptor system[97].

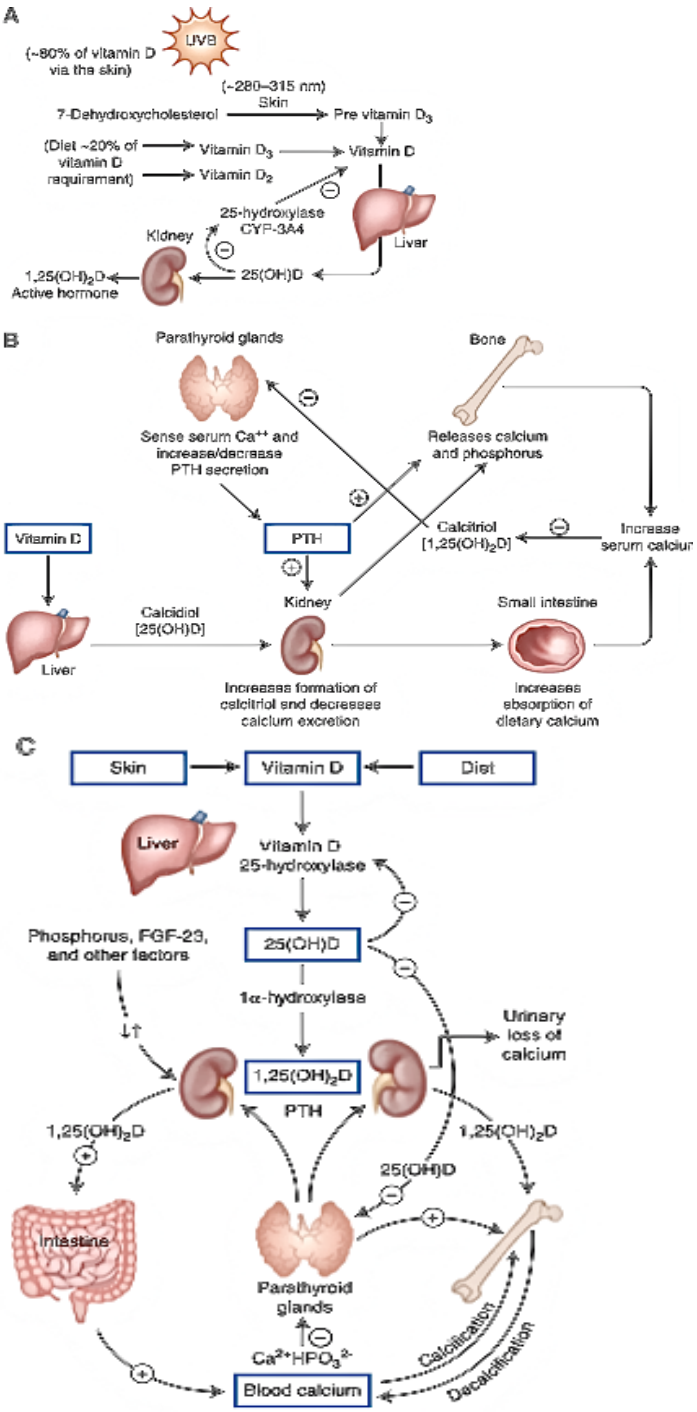

Figure 1. A The final common pathways of generation of 25 (OH)D and 1,25(OH) 2D, the feedback loop, and the roles of skin, liver, and the kidney. Eighty percent of the vitamin D requirements of humans is generated through UVB rays after skin exposure to sunlight. **B** Illustrate the physiologic role of PTH in the maintenance of serum calcium level. Key target organs for PTH; bone, kidney and intestine, and their feedback interactions with calcium are illustrated**. C** Feedback hormonal control of calcium metabolism. Low serum ionized calcium enhances the secretion of PTH from parathyroid glands. Subsequently, PTH increases the synthesis of 1,25(OH)2D, stimulates calcium absorption from the intestine, and mobilizes calcium from the skeleton to maintain normocalcemia, and negatively regulates PTH synthesis and release. FGF-23= fibroblast growth factor-23; PTH= parathyroid hormone; UVB= ultraviolet B. Adapted from *Curr Osteoporos Rep*, 2012[82].

2.2. Sources

Vitamin D is a particular micronutrient, since it can be obtain not only form food consumption, but also by skin synthesis throughout UVB rays, which, in fact, are its main source, contributing with 80% compared with 20% that come from food[76].

Determining adequate levels for vitamin D is rather complex. In fact, vitamin D is known as the "sunshine vitamin" because modest exposure to sunlight should be sufficient for most people produce their own vitamin D using ultraviolet light and cholesterol in the skin.

Vitamin D_3 is synthesized in human skin from 7-dehydrocholesterol following exposure to ultraviolet B (UVB) radiation with wavelength 290 to 320 nm. Its production in skin is a function of the amount of UVB radiation reaching the dermis as well as the availability of 7-dehydrocholesterol. As such, the level of synthesis varies greatly from person to person and is influenced by a number of factors, which include season of the year, skin pigmentation, latitude, use of sunscreen, clothing, and amount of skin exposed. Even though brief and casual exposer of the face, arms, and hands to sunlight could contribute to the decreasing lack of vitamin D, people are justifiably advised against sun exposure to reduce the risk of skin cancer[98]. Age is also a factor, as synthesis of vitamin D declines with increasing age, due in part to a fall in 7-dehydrocholesterol levels and also to alterations in skin morphology[99].

Contrary to excessive oral vitamin D intake, toxic levels of vitamin D do not occur from prolonged sun exposure. In fact, thermal activation of previtamin D_3 in the skin gives rise to multiple non–vitamin D forms, such as lumisterol, tachysterol and others, which limit the formation of Vitamin D_3 itself. Besides, vitamin D_3 can also be converted to nonactive forms[100, 101].

Vitamin D serum levels are determined by the measurement of 25-hydroxyvitamin-D, which reflects vitamin D input from both skin absorption of sun and dietary intake. Normative values are subject to debate, but most experts use the following guidelines: levels less than 20 ng/ml is defined as deficiency; 21 to 29 ng/ml is relative insufficiency; 30 ng or greater is sufficient; greater than 150 ng/ml is intoxication[76].

Dietary sources of vitamin D include food and supplements. There are a few naturally occurring food sources of vitamin D, which include fatty fish, fish liver oil, and egg yolk. Some foods are, however, fortified with vitamin D, such as fortified milk, margarine and cereal[102] (table 3). In recent years, dietary supplements containing vitamin D have become more common and have been more frequently

consumed. The form of vitamin D used in supplement products can be either vitamin D_2 or vitamin D_3[82].

Table 3. Major food sources of vitamin D.

Selected food sources	International Units (IU) per serving
Cod liver oil (1 tablespoon)	1360
Swordfish, cooked (3 ounces)	566
Salmon, cooked (3 ounces)	447
Tuna fish, canned in water (3 ounces)	154
Egg yolk (one)	40

Adapted from *The National Institutes of Health*, June 2011
http://ods.od.nih.gov/factsheets/VitaminD-HealthProfessional/

Vitamin D levels in the diet are expressed in International Units (IU), but can also be expressed in micrograms (µg). The biological activity of 1 µg of vitamin D is equivalent to 40 IU (table 4).

Table 4. Conversions used in reference to Vitamin D[90].

IU = International Unit is a measurement based on biological activity or effect; 1 IU of vitamin D is defined as the activity of 0.025 µg of cholecalciferol in bioassays with rats and chicks.

Conversions for Vitamin D3:

[sources] 40 IU = 1 µg

[serum] 2.5 nmol/L = 1 ng/mL

Although some aspects of vitamin D nutrition and physiology have been found to differ with life stage, most of the functions of vitamin D are quite consistent across life stages from infancy and childhood, to adolescence, adulthood, and old age[90]. Therefore, the recommendations from the *Institute of Medicine* (IOM) 2011, which assumed minimal sun exposure when establishing the DRIs for vitamin D, determined that 600 IUs of vitamin D per day meets the needs of almost everyone, except people aged 71 and older, who may need as much as 800 IUs per day due to potential changes in people's bodies as they age[90].

The Estimated Average Requirements (EARs), Recommended Dietary Allowances (RDAs), and Adequate Intakes (AIs) for vitamin D by life stage group are shown in table 5. The identical EARs across age groups are notable, reflecting the concordance of serum 25OHD levels with the integrated bone health outcomes as well as the lack of an age effect on the simulated dose-response[90].

For very young children in this life stage group, no data is available to link vitamin D directly to measures related to bone health outcomes[90]. Regarding adolescence ensuring normal, healthy bone accrual is central to the DRI values. The requirement distribution developed using serum 25OHD concentrations and the intakes estimated to achieve such concentrations are the basis for the reference values[90]. Thus, IOM indicates that an intake of vitamin D of 400 IU/day achieves serum concentrations of 40 nmol/L, and this intake is therefore set as the EAR for persons 1 to 3 years, 4 to 8 years, 9 to 13 years, and 14 to 18 years of age. As this requirement distribution appears to be normally distributed, the assumption of another 30 percent to cover nearly all the population (i.e., 97.5 percent) is appropriate and consistent with a serum 25OHD level of approximately 50 nmol/L as the target for an RDA value. Based on the same analysis relating serum 25OHD levels to intake, an intake of 600 IU/day is set as the RDA[90].

Table 5. Vitamin D Dietary Reference Intakes (DRIs) for Adequacy (amount/day).

Life Stage Group	AI	EAR	RDA
Infants			
0 to 6 mo	400 IU (10mcg)	-	-
6 to 12 mo	400 IU (10mcg)	-	-
Children			
1-3 y	-	400 IU (10mcg)	600 IU (15mcg)
4-8 y	-	400 IU (10mcg)	600 IU (15mcg)
Adolescents (both genders)	-		
9-13 y	-	400 IU (10mcg)	600 IU (15mcg)
14-18 y		400 IU (10mcg)	600 IU (15mcg)
Adults (both genders)			
19-30 y	-	400 IU (10mcg)	600 IU (15mcg)
31-50 y	-	400 IU (10mcg)	600 IU (15mcg)
51-70 y	-	400 IU (10mcg)	600 IU (15mcg)
> 70 y	-	400 IU (10mcg)	800 IU (20mcg)
Pregnancy			
14-18 y	-	400 IU (10mcg)	600 IU (15mcg)
19-30 y	-	400 IU (10mcg)	600 IU (15mcg)
31-50 y	-	400 IU (10mcg)	600 IU (15mcg)
Lactation	-		
14-18 y	-	400 IU (10mcg)	600 IU (15mcg)
19-30 y	-	400 IU (10mcg)	600 IU (15mcg)
31-50 y	-	400 IU (10mcg)	600 IU (15mcg)

AI = Adequate Intake; EAR = Estimated Average Requirement; IU = International Unit;
RDA = Recommended Dietary Allowance.
Adapted from *The Institute of Medicine* 2011.

In recent years numerous position statements and clinical practice guidelines have been published to define the optimal vitamin D status and the health outcomes associated with its alteration[103, 104]. Different recommendations on dietary intakes needed to reach and maintain sufficient 25(OH)D levels have been proposed as well[90, 105-108]. In this context, the publication of the most authoritative report on these issues for the general population, The Institute of Medicine concluded that 25(OH)D levels above 20ng/ml are needed for good bone health for almost individuals (97,5% of the population), while a level of 16ng/ml (40nmol/l) meets the need of approximately half the population[90]. According to IOM, higher levels of 25(OH)D have not been consistently shown to confer greater benefits, in turn challenging the concept that "more is better". It highlights the need to take into account several factors when interpreting the results of current literature, which include the difficulty to distinguish the sole effect of vitamin D as the majority of intervention trials co-administered calcium; the difficulty to exactly measure the relative contribution of sunlight exposure, foods fortification and multivitamins intake; the lack of randomized trials assessing the effect of vitamin D supplementation on health outcomes other than bone and the complexity to compare studies utilizing different 25(OH)D assays[109-112].

In fact, the primary health outcomes of vitamin D intake utilized to define vitamin D sufficiency are those related to skeletal health. Concerning the other possible benefits of vitamin D, IOM concluded that existing data is not sufficient to support the recommendation of vitamin D supplementation to reduce the risk of extra-skeletal acute and/or chronic diseases[90].

The definition of vitamin D deficiency, insufficiency and sufficiency is currently challenging as an overall consensus is still lacking[82, 113, 114]. It represents a crucial issue, since the identification of different thresholds defining vitamin D status has varying implications in clinical practice. First, the worldwide prevalence of low vitamin D status is highly varying according to the level of 25(OH)D utilized to define sufficiency. Consequently, the choice to initiate vitamin D supplementation may change, as well as the goals of therapy, the dosing strategy, and the decision about who should be screened, if necessary, and how often[115].

In general, for every 100 IU (2,5µg) of vitamin D taken in, 25(OH)D levels increase of about 1ng/ml, but with a huge inter-individual variability. Several factors may account for such variability: the initial 25(OH)D concentration, patient's weigh, adequacy of dose according to compliance, the type of vitamin administered (D_2 or D_3), renal function, and genetic factors. The variability in absorption, the inaccuracy

of 25(OH)D assessment, as well as unknown factors also probably contribute to the variability of the dose-response relationship[116-118].

Controversy also exists on whether supplementation should be given daily or intermittently (e.g. weekly, monthly, once a year). It has been shown that circulating levels of 25(OH)D increase similarly when oral vitamin D is given daily, weekly or monthly, proving that the total amount is identical. However, it must be recognized that a universal guideline does not exist, most likely the result of great disparity among countries in the availability of vitamin D supplements[119].

The immediate aim of treatment should be quick normalization of 25(OH)D levels, as well as vitamin D stores, which can be accomplished with an initial period of high-dose vitamin D. An intermittent high-dose therapy is an interesting option to avoid non-adherence to treatment, although a regimen of regular low-dose is a reasonable alternative[120]. Studies comparing the two different regimens reported inconsistent results, and both high-dose (dosing interval < 2 months) and more regular low-dose seem to offer similar efficacy[121-123]. The maximum safe bolus remains uncertain. A number of papers reported that a single oral dose of 300,000-600,000IU of D_2 or D_3 rapidly enhances serum 25(OH)D and reduces PTH levels in patients with deficiency[124, 125]. However, the study by Sanders and co-workers showing that 500,000IU oral dose of cholecalciferol increased the risk of falls and fractures among older women deserves attention[126]. Another trial reported that 300,000 IU of ergocalciferol given intramuscularly for 3 years to elderly people during fall season did increase fracture risk[127]. No plausible biological explanation has been given for these results, whose interpretation remains merely speculative. However, these papers raise the possibility that infrequent high doses of vitamin D status may be unsafe, counteracting any possible beneficial outcome. The rate and magnitude of the increase in serum 25(OH)D levels may be critical, as well as at which time points 25(OH)D concentration should be measured after dosing. On the other hand, it is undeniable that, on a population basis, the utilisation of intermittent doses could aim to overcome the problem of compliance[128-130].

Once vitamin D stores are replete, a maintenance dose of 800 to 2000IU (20 to 50 μg) should be recommended. In particular, long-term supplementation has to be encouraged in special groups that are at high risk of deficiency. At this regard, many experts have questioned the IOM recommendations since, in the absence of sun exposure and dietary input, a daily dose of 600 IU (15 μg) of vitamin D will not maintain blood 25(OH)D levels, even at 20ng/ml[131, 132]. Hence, higher doses may be probably necessary to achieve an optimal vitamin D status.

Regarding the type of vitamin D supplementation, current evidence suggests that ergocalciferol has a considerably lower efficacy than cholecalciferol in raising circulating 25(OH)D level[120]. This difference between the two calciferols relates to several factors: the different affinity for the vitamin D binding protein and VDR, the different calciferols relates to several factors: the different affinity for the vitamin D binding protein and VDR, the different affinity as substrate for hepatic 25-hydroxilase, a possible difference in the 24-hydroxilation rate. In fact, the metabolism of vitamin D involves 24-hydroxilation in the kidney to form $1,24,25(OH)_3D$. This step is critical, since once $1,24,25(OH)D_3D_2$ has been formed, ergocalciferol has been deactivated and, therefore, is irretrievable. On the contrary, the $1,24,25(OH)_3D_3$ still binds to VDR [≈ 40% more than $1,25(OH)D_2D_3$] and must undergo additional side chain oxidation to be biologically deactivated. This additional step gives a vast advantage and potential for cholecalciferol to remain biologically active and, thus, maintain vitamin D status. Available data also document the higher efficacy of cholecalciferol, regardless the frequency of administration (small doses or in larger and more infrequent bolus)[133-135].

When considering the route of administration, both cholecalciferol and ergocalciferol are available as oral or intramuscular (IM) preparations. In general, oral administration is more physiological and leads to a rapid increase in serum 25(OH)D levels within 3 days[124]. With IM injection a gradual increase in serum 25(OH)D levels was observed, thus demonstrating delayed serum 25(OH)D response[124]. This phenomenon is probably due to the sequestration of vitamin D in the muscle and fat, where it is gradually released. It has been hypothesized that this pharmacokinetic profile potentially allows IM preparations to overcome the fluctuation of serum 25(OH)D levels following high oral bolus[136]. However, this point has not been definitively clarified. On the other hand, intramuscular preparations may have specific indications, in particular for intermittent (once or twice-yearly) high-dose regimens[137].

As aforementioned, vitamin D intoxication is rather unusual. In fact, the potential harm of vitamin D may only come from the excessive ingestion of supplements, as after intensive solar UVB irradiation the skin synthesis of vitamin D is self-regulated as inactive metabolites are produced, available data demonstrating that skin synthesis of up to 10,000IU (250 μg) daily is safe. Moreover, the contribution of dietary sources is usually about 10-20% being intoxication from this source nearly impossible[138, 139]. The condition of hypervitaminoses D leads to hypercalcemia and eventually to soft tissue calcification and resultant renal and cardiovascular damage[85].

A number of studies linked the amount of vitamin D intake to the achieved 25(OH)D serum levels, in order to establish a threshold for intoxication. It has been reported that an intake of 10,000IU/d (250g/d) of vitamin D is not harmful, which correspond to a 25(OH)D serum level of about 88ng/ml (220nmol/l)[140]. Nevertheless, the IOM recently set the upper safe level of 25(OH)D at about 50ng/ml (125nmol/l), on the basis of observational studies showing an U-shaped association between circulating 25(OH)D and some clinical outcomes (frailty, all cause mortality, cancer, falls and fractures)[141]. 25(OH)D serum levels beyond this limit are considered potentially harmful. However, this threshold seems to be very conservative, especially if we take into consideration many published studies showing that doses around 4000IU/d (100 µg/d) of vitamin D are safe, even in the long-term treatment. The 25(OH)D serum level achieved in these studies was between 30 and 64ng/ml (75 and 160 nmol/l), and it was not accompanied by any clinical sign of intoxication[139].

2.3. Functions an physiological actions

Vitamin D has both genomic and nongenomic functions. For the genomic functions, 1,25(OH)2D interacts with nuclear vitamin D receptors to influence gene transcription[86]. Nuclear receptors for 1,25(OH)2D have been identified in over 30 cell types, including bone, intestine, kidney, lung, muscle and skin. For the nongenomic functions, 1,25(OH)2D acts like a steroid hormone, working through activation of signal transduction pathways linked to vitamin D receptors on cell membranes. Major sites of action include intestine, bone, parathyroid, liver and pancreatic beta cells. Biological actions include increases in intestinal calcium absorption, transcellular calcium flux and opening gated calcium channels allowing calcium uptake into cells such as osteoblasts and skeletal muscle[86].

The dominant function of vitamin D in its hormonal form (calcitriol or 1,25-dihydroxyvitamin D) is the elevation of plasma calcium and phosphate levels, which are required for mineralization of bone[142]. Furthermore, the elevation of plasma calcium to normal levels is also required for the functioning of the neuromuscular junction as well as vasodilatation, nerve transmission, and hormonal secretion[143].

Calcitriol, functioning as part of the endocrine system for maintaining serum calcium levels, elevates plasma ionized calcium levels to the normal range by three different mechanisms.

The first mechanism is the well-established role of calcitriol in stimulating intestinal calcium absorption throughout the entire length of the intestine, although its greatest activity is in the duodenum and jejunum. It is clear that calcitriol directly stimulates intestinal calcium and, independently, phosphate absorption. In the second mechanism, calcitriol plays an essential role in the mobilization of calcium from bone, a process requiring PTH. It induces the formation and activation of the osteoclast to function in the mobilization of calcium from bone. In short, calcitriol facilitates the formation of osteoclasts by stimulating the secretion of a protein called receptor activator for nuclear factor κ B (RANK) ligand, which, in turn, is responsible for osteoclastogenesis and bone resorption. In the third mechanism, calcitriol together with PTH stimulates the renal distal tubule reabsorption of calcium, ensuring retention of calcium by the kidney when calcium is needed[144, 145].

Therefore, overall, calcitriol acts on the intestine, bone, and kidney as described above, to elevate serum calcium levels, closing the calcium loop[146]. As serum calcium levels rise, PTH secretion drops. If serum calcium levels become too high, the parafollicular cells ("C" cells) of the thyroid secrete calcitonin, which blocks calcium resorption from bone and helps to keep calcium levels in the normal range. Calcitriol, through its receptor, the VDR, suppresses parathyroid gene expression and parathyroid cell proliferation, providing important feedback loops that reinforce the direct action of increased serum calcium levels[146].

In addition to the aforementioned mechanisms, it was recently recognized that the VDR is present in the nucleus of many tissues that are not involved in the regulation of calcium and phosphate metabolism[147]. The role of this paracrine production of 1,25(OH)D in these tissues is not well understood, but a variety of *in vitro* studies indicated that this process may be involved in a wide range of physiologic functions, including regulation of cytokines, inflammatory and/or fibrotic pathways, the renin-angiotensin system, vascular and cardiac cell function, immune response modulation, cell growth and differentiation, and others[88, 148, 149]. For example, the VDR has been clearly described in epidermal keratinocytes, in activated T cells of the immune system, in antigen-presenting cells, in macrophages and monocytes, and in cytotoxic T cells[150]. Gene array studies in many cells and tissues show that calcitriol regulates several hundred genes throughout the body or as much as 5 percent of the human genome, though exactly how calcitriol functions in these tissues and the physiological consequences are not clearly known[151].

Several of the biologic pathways through which the effects of 1,25(OH)D are mediated remain poorly understood but may account for its role in cardiovascular health.

3. Vitamin D and the metabolic syndrome

Low vitamin D status is being increasingly recognized as widespread in all life stages, even in sunny climates[81, 152].

Beside its role in bone metabolism, the possible importance of vitamin D deficiency as a novel risk factor for various chronic diseases has gained more interest. One area of recent study has been the investigation of the association between vitamin D status and the *metabolic syndrome*[89, 153].

Although the underlying biological causes are not fully understood, the association of 25OHD with cardiovascular pathology is suggested to be driven by various mechanisms: apart from a potential direct impact on cardiomyocytes and myocardial diseases[83] it has been suggested that 25OHD indirectly modifies CVD risk[154] by its association with cardiovascular risk factors like diabetes[155], hypertension[156], or cholesterol level[157].

Epidemiological studies have suggested that low vitamin D status is associated to the development of the metabolic syndrome. Ford *et al.* reported the association in the NHANES III, a nationally representative cross-sectional survey of the noninstitutionalized population in the United States carried out during 1988–1994, between vitamin D status and the metabolic syndrome in 8421 men and nonpregnant women who were 20 years of age or older. The mean serum 25-hydroxyvitamin D concentration in those with metabolic syndrome was significantly lower (67.1 nmol/L) than that in subjects without metabolic syndrome, which in turn had a mean serum 25-hydroxyvitamin D concentration of 75.9 nmol/L. When compared by quintiles of serum 25-hydroxyvitamin D, they also found a significant inverse association with the metabolic syndrome and some of the individual components (abdominal adiposity, hypertriglyceridemia, and hyperglycaemia)[158].

In another study, Liu *et al.* analysed data from 10,066 women 45 years of age or older who participated in the Women's Health Study, and observed a median calcium intake of 857 mg/d and a median vitamin D intake of 266 IU/d. Interestingly, an inverse association between dietary calcium and metabolic syndrome was observed within each tertile of dietary vitamin D intake without significant interaction, suggesting that calcium intake was associated with a lower prevalence of the metabolic syndrome than vitamin D alone. However, the authors pointed out that the null findings for vitamin D intake might be attributed to an inadequate reflection of vitamin D intake on overall vitamin D status due to the lack of information on sun exposure. [159]

Less attention has been given to adolescence. Reis *et al.* conducted a cross-sectional analysis of 3577 adolescents who participated in the 2001-2004 NHANES and concluded that low serum vitamin D in US adolescents is strongly associated with hypertension, hyperglycaemia, and metabolic syndrome, independent of adiposity[160], which is, to best of our knowledge the only study that evaluated the association between vitamin D status and the metabolic syndrome regarding the period of adolescence.

Even though the mechanisms are not fully comprehended, in the following it will be reviewed the current knowledge on the mechanisms by which vitamin D may influence some of the risk factors of the MetS.

3.1. Vitamin D and obesity

The prevalence of obesity has increased in the past decades and it is presently the most common and costly nutritional problem. For example, in the United States, one-third of the population is affected by obesity, according to the National Health and Nutrition Examination Survey (NHANES)[161]. Vitamin D deficiency is another increasingly prevalent public health concern in developed countries, and there is evidence that vitamin D metabolism, storage, and action influence and are influenced by adiposity[162].

Although the underlying explanations and direction of causality are unclear, observational studies have reported an increased risk in vitamin D deficiency in those who are obese. The probable explanation is that vitamin D is stored in the adipose tissue and, therefore, the association is that the larger storage capacity for vitamin D in obese individuals leads to lower circulating 25-hydroxyvitamin D [25(OH)D] concentrations, a marker for nutritional status[163].

As previously mentioned adipose tissue stores of vitamin D probably represent “non-specific” stores sequestered because of the hydrophobic nature of vitamin D, but the extent to which the processes of accumulation or mobilization are regulated by normal physiological mechanisms remains unknown.

Wortsman *et al.* concluded that in obese subjects, vitamin D was stored in adipose tissue and not released when needed[164]. Blum *et al.* found that, in elderly subjects supplemented with 700 IU of vitamin D per day, for every additional 15 kg of weight above “normal” at baseline, the mean adjusted change in 25OHD level was approximately 10 nmol/L lower after 1 year of supplementation[165]. The authors estimated that in order for subjects with body mass indexes (BMIs) above the normal

range to obtain an increase in serum 25OHD level similar to that of subjects with weight in the normal range, an additional 17 percent increase in vitamin D above the administered dose of 700 IU/day would be needed for every 10 kg increase in body weight above baseline in their study population.

Vimaleswaran *et al.* demonstrated that the association between BMI and lower 25(OH)D concentrations in Caucasian populations from North America and Europe can be seen across different age groups and in both men and women. They also showed that higher BMI leads to lower vitamin D status, highlighting the role of obesity as a possible causal risk factor for the development of vitamin D deficiency[163].

The implication of these studies is that vitamin D deposited in fat tissue is not readily available, and obese individuals may require larger than usual doses of vitamin D supplements to achieve a serum 25OHD level comparable to that of their normal weight counterparts[90].

3.2. Vitamin D and blood pressure

Growing evidence points to the existence of an association between vitamin D and blood pressure[166]. In knockout mice was shown that the absence of vitamin D receptor activation leads to tonic upregulation of the renin-angiotensin system, with the development of hypertension and left ventricular hypertrophy[148, 167].

Indeed, evidence suggests that vitamin D may regulate blood pressure via regulating the renin-angiotensin system, which is a regulatory cascade that plays an essential role in the regulation of blood pressure, electrolyte and volume homeostasis. The main function of renin is to cleave angiotensin I (Ang), from angiotensinogen. Ang I is then converted to Ang II by the angiotensinogen-converting enzyme (ACE), which primarily resides in endothelial cells in blood vessels. In fact, Ang II is the central effector of the RAS. Through interacting with Ang II receptors in different tissues, including the brain, heart, kidney, adrenal glands and peripheral vasculature, Ang II exerts diverse physiological responses that influence the electrolyte and extracellular volume balance and blood pressure. Inappropriate stimulation of the RAS has been associated with hypertension.

In their work, Li and colleagues, concluded that, under normal physiological conditions, in addition to maintaining the blood calcium concentration, 1,25(OH)D antagonizes other renin-stimulating factors to maintain an appropriate renin level in the body. Therefore, the disruption of the Vitamin D signaling pathway should lead to

a deregulated stimulation of renin synthesis and consequently to an increase on blood pressure, whereas an increase in serum 1,25(OH)D levels should lead to renin suppression[148].

Data from NHANES III also showed an inverse association between blood pressure and vitamin D concentration. Martins *et al* verified that the adjusted prevalence of hypertension in adults was 30% higher in the lowest quartile compared to the highest quartile of serum 25(OH)D[155].

Forman *et al*. prospectively investigated the independent association between plasma 25(OH)D levels and risk of incident hypertension. Two prospective cohort studies that included 613 men from the Health Professionals' Follow-Up Study and 1198 women from the Nurses' Health Study with measured 25(OH)D levels were followed for 4 to 8 years. After this follow-up, the risk of incident hypertension among those whose measured plasma 25(OH)D levels were <15ng/mL (compared with those whose levels were >30 ng/mL) was 6.13 in men and 2.67 in women[156].

Nevertheless, there have been studies that contradict this hypothesis as well. A large prospective study by Forman *et al.* in 2005 found no association between vitamin D intake from diet and supplements and the risk of incident hypertension[168]. Likewise the randomized double-blind trial by Scragg *et al.* with a vitamin D supplementation at a single dose of 2.5mg in winter months did not show any significant decrease in blood pressure after 5 weeks when compared to placebo[169].

The conflict between the above-mentioned results suggests that the effect of vitamin D on blood pressure could be due to an indirect effect rather than a direct effect. In support of this hypothesis, some authors[170-172] identified a positive correlation between blood parathyroid hormone, which is usually high in hypovitaminosis D and high blood pressure, and this effect could be mediated through the action of parathyroid hormone on vascular smooth muscle cells of blood vessels. However, to clear this issue, using knockout mice Kong *et al.* verified the lack of involvement of parathyroid hormone in the suppression of blood pressure by vitamin D, showing that suppression of renin expression by 1,25-dihydroxyvitamin D in vivo is independent of parathyroid hormone and calcium[173].

3.3. Vitamin D and glucose/insulin metabolism

Interest in studying the relationship between vitamin D, glucose and insulin metabolism has been recently intensified due to the increase incidence of diabetes worldwide[2].

Recent studies have shown a positive correlation between vitamin D deficiency and both diabetes[155] [174, 175] and insulin resistance[174, 176], suggesting that high vitamin D status offers protection from diabetes[177, 178].

Animal research supports the role for vitamin D in glucose and insulin metabolism. Studies have shown that vitamin D deficiency inhibits rat pancreatic insulin synthesis and secretion, which can be corrected by supplying the vitamin[179, 180]. In diabetic rats, 1,25-OH vitamin D_3 injection increased insulin gene expression in liver and adipose tissue without affecting the insulin receptor affinity to insulin[181]. Moreover, poor vitamin D status has been associated with β-cell dysfunction in both diabetic and non-diabetic subjects[182].

1,25(OH)2D, via VDR-mediated modulation of calbindin expression, appears to control intracellular calcium flux in the pancreatic islet cells, which in turn affects insulin release[183].

Observational studies also support these results. A meta-analysis of published work in this area revealed that vitamin D may play a role in the development of type-2 diabetes and in combination with adequate calcium intake vitamin D may favourably influence glucose metabolism[184]. Reis *et al.*, in their analysis of NHANES 2001–2004 data on adolescents, also showed an inverse relationship between 25(OH)D levels and plasma glucose concentrations[160].

3.4. Vitamin D and dyslipidaemia

The roles of vitamin D in dyslipidaemia have received much less attention from investigators compared to previously discussed risk factors[153].

In a retrospective study, Guasch *et al.* observed 316 patients who had attended the obesity clinics in a Spanish hospital and categorized them by degree of adiposity, presence of MetS, and other comorbidities. They found that the prevalence of hyperparathyroidism increased from 12% in non-obese to 47.5% in morbidly obese individuals with BMI>50 kg/m^2 and low plasma 25(OH)D and high PTH concentrations were associated with an increased risk of MetS and atherogenic dyslipidaemia, supporting the idea that a possible contribution of plasma 25(OH)D to the pathogenesis of hypertriglyceridemia and dyslipidaemia through inflammation[185].

Dyslipidaemia is a major contributing factor for atherosclerosis. Along with inflammation, it causes cardiovascular damage. Dyslipidaemia and a proinflammatory-prothrombotic state are characteristics of the metabolic syndrome.

There is compelling evidence that vitamin D status, and specifically 1,25-dihydroxyvitamin D, can affect cytokine production and immunity. The hormonal form of vitamin D can inhibit the production of proinflammatory cytokines, including interleukin (IL)-1, IL-2, IL-6, tumor necrosis factor-α, and others, likely via the vitamin D receptor expressed in monocytes and activated T lymphocytes[186]. Although it is well known that vitamin D plays a role in immunity, there is little evidence that vitamin D status is connected to the proinflammatory or prothrombotic components of the metabolic syndrome. Low circulating 25-hydroxyvitamin D levels have been noted in some patients with cardiovascular disease and dyslipidaemia, and hypovitaminosis D has been associated with increased total serum cholesterol concentration[187, 188].

Nonetheless, there is little evidence suggesting a likely mechanism by which vitamin D status could affect the development of dyslipidaemia[162].

4. The importance of adolescence

Adolescence is one of the most exciting yet challenging periods in human development. This period, which comprises young people between the ages of 10 and 19 years, involves enormous physiological, psychological and cognitive transformation, characterized by prompt growth and maturation during which a child becomes a young adult[189].

Early adolescence involves the rapid biological changes and their adjustment. Changes in cognitive and emotional functioning allow teens to become more independent as they mature. Peer influence and acceptance may become more important than family values, creating periods of conflict between teens and parents. Late adolescence is characterized by increased interest in future choices concerning adult life. It is a time when teenagers begin to emancipate and to consider what are their own ideals in order to start building their life goals[190].

Adolescents are often thought of as a healthy group. By the second decade of life they have survived the diseases of early childhood, have a low incidence of infections compared with younger children and the overwhelming health problems associated with ageing are still many years away. Nevertheless, the incidence of some diseases once considered to affect mainly the adult population, have been increasing in this age group[191].

Some of the diseases that affect the adulthood have their roots in adolescence. Even though cardiovascular disease events occur most frequently during or after the fifth decade of life, pathologic evidence suggests that precursors of cardiovascular disease originate in childhood[2, 34]. Longitudinal studies have shown that risk factor levels in young adulthood track into and predict the occurrence of cardiovascular disease in adulthood[2]. In fact, there is evidence that when exposed to risk factors, changes of atherosclerosis within blood vessels begin in the first decade of life as fatty streaks and plaques, which have been shown to regress through modification of behavioural risk factors[12].

Adolescence may, thus, be the key opportunity to intervene and prepare for a healthy adult life, preventing the onset of behaviours that will affect health and well being in the future, given the difficulty for people to change their behaviours in later life. Consequently, to know the relationship between vitamin D and cardiovascular risk factors in this age group, can help to better understand whether this micronutrient may be an added value for the ongoing battle against the development of cardiovascular disease in adulthood.

Recently, IOM has increased the previous value for adequate intake for vitamin D (5μg) to 10 μg EAR for children 1 to 18 years of age for both genders[90], which highlights the increasing confidence of its beneficial role in public health.

A significant association between vitamin D and cardiovascular risk factors in youth may, therefore, suggest that the successful repletion of vitamin D has the potential to improve the cardiovascular risk profile during childhood and adolescence and to lower the risk of developing cardiovascular disease in adulthood.

OBJECTIVE

Using the baseline information obtained after assembling the “Epidemiological Health Investigation of Teenagers in Porto” (EPITeen) cohort study, this research aimed to evaluate the vitamin D intake and its association to metabolic syndrome and its components in 13-year-old adolescents.

MANUSCRIPT

Vitamin D intake and metabolic syndrome among 13-year-old Portuguese adolescents

Abstract

Background: Growing evidence suggests a possible association between low vitamin D and increased cardiovascular risk. However, research regarding the period of adolescence is scarce.

Objective: To evaluate the association of vitamin D intake with metabolic syndrome and its components in 13-year-old adolescents.

Methods: We conducted a cross-sectional analysis of 1033 adolescents aged 13-years-old, born in 1990 and enrolled at public and private schools in Porto, during the 2003/2004 school year. Self-administered questionnaires were applied and vitamin D intake was assessed by a food frequency questionnaire. Anthropometric assessment, blood pressure measurement and fasting blood sample collection were performed. Metabolic syndrome components were defined according to the National Cholesterol Education Program Adult Treatment Panel III definition modified for age. Logistic regression was performed in order to estimate the association between vitamin D intake and metabolic syndrome components.

Results: Median (P25-P75) vitamin D intake was 4.11 (2.88-5.60) µg; it was higher among adolescents who reported the use of vitamin supplements and lower in those classified as obese or abdominally obese. Metabolic syndrome prevalence was 13.2%. The adjusted odds ratio (95% confidence interval) increased with the decrease of vitamin D intake until 1.22 (0.84-1.78) for low HDL cholesterol and 1.78 (1.00-3.17) for high BMI.

Conclusions: Notwithstanding the extremely low vitamin D intake, an inverse relation with high BMI and low HDL cholesterol was observed, though without statistical significance. No trend was found for metabolic syndrome.

Key words: Vitamin D, metabolic syndrome, adolescents.

Introduction

Cardiovascular disease (CVD) remain the leading cause of death and disability worldwide[1]. Although the events occur most frequently during or after the fifth decade of life, longitudinal studies have shown that risk factors persist from childhood into adulthood in a large percentage of cases (25-60%)[2, 3] and predict the occurrence of CVD in later life[4].

Besides the contribution to the rise in obesity, the "obesogenic" environment that has been installed in society during the last decades has also contributed to the increase in metabolic/physiologic changes [5]: raised blood pressure (hypertension); overweight/obesity; impaired glucose and raised blood lipids (dyslipidaemia). The clustering of these risk factors, which is associated with insulin resistance and co-occur more often than it might be expected by chance, is called the *Metabolic Syndrome* (MetS)[6].

Vitamin D, first identified in the 20th century and known as "the sunshine vitamin" due to its particularity of a nutrient that can be synthesized by the human body through the action of sunlight, is now recognized as a pro-hormone. For decades, a great deal of attention has been justifiably placed on understanding the consequences of vitamin D deficiency on bone health[7-9]. However, recent studies have broadened the horizon, suggesting that vitamin D levels may play an important role in cardiovascular disease[10-13].

Vitamin D biological actions involve regulation of gene expression at the transcriptional level, and are mediated through binding to a vitamin D receptor (VDR), located primarily in the nuclei of target cells. The acknowledgement that VDR is found fairly present throughout the body in tissues not involved with calcium and phosphate homeostasis, implies that the presence of VDRs in these tissues may play a more general role than it was previously thought[14, 15].

The mechanisms by which vitamin D may be linked to metabolic syndrome involve the blunting effect of advanced glycation end products on endothelial cells, which contribute to the increased arterial stiffness and endothelial dysfunction observed in individuals deficient in vitamin D. Additionally, vitamin D may also exert protective effects on the vessel walls by inhibition of macrophage to foam cell formation and via its anti-inflammatory effects. Lastly, vitamin D sufficiency has been associated with downregulation of the renin-angiotensin-aldosterone system[16].

Despite the emerging evidence of an association between low circulating concentrations of vitamin D and the prevalence of metabolic syndrome[17, 18],

epidemiologic evidence regarding vitamin D intake to the metabolic syndrome is limited. Notwithstanding, the *Institute of Medicine* (IOM) has increased the previous value for adequate intake for vitamin D (5µg) to 10 µg EAR[19], highlighting the increasing confidence of its beneficial role in public health.

Once the metabolic syndrome and its components often begin to be established throughout adolescence[20], this is a period when a dietary intervention to prevent the occurrence of disease and its consequences may conduct to greater benefits[21].

The present study aimed at examining the association of vitamin D intake with metabolic syndrome and its components in 13-year-old adolescents.

Methods

Eligible participants were urban adolescents, members of the *Epidemiological Health Investigation of Teenagers* in Porto (EPITeen). As reported elsewhere[22], we evaluated adolescents born in 1990, who were enrolled at public and private schools in Porto, Portugal, during the 2003/2004 school year. Data were collected using two self-administered questionnaires, one fulfilled at home and another at school. The home questionnaire inquired about demographic, social, behavioural and clinical characteristics of the children and the family. A physical examination was performed at school, by a team of experienced nurses, nutritionists and physicians. During the research team visit, children answered an additional questionnaire comprising further information on physical activity, smoking and alcohol intake.

Assessment of vitamin D intake

As part of the home questionnaire, food intake was recorded using a food frequency questionnaire (FFQ) regarding the previous 12 months, fulfilled by the adolescents at home with the help of their parents or legal guardians. The FFQ was designed according to *Willett* and colleagues[23] and adapted for the Portuguese population, according to dietary data available for our country, namely the Portuguese food balance sheets and other specific studies[24]. Foods with similar nutrient composition were grouped together as a single food item. The questionnaire was validated for the adult population by comparison with four 7 daily food records (each one in a different season of the year)[24]. The FFQ was then adapted for adolescents by including foods more frequently eaten by this age group. The adolescents' version comprised ninety-one food items or beverage categories and a frequency section with nine

possible responses ranging from never to six or more times daily. It also included an open-ended section for foods not listed in the questionnaire, but eaten at least once weekly.

Food intake data were obtained by multiplying the frequency of consumption of each food item by the nutrient content of the specified portion size. Seasonal variation of food consumption was also considered according to participants' replies. To estimate nutrient intake from the evaluated food intake, we used the software *Food Processor Plus*® (ESHA Research, Salem, OR, USA) based on values from the US Department of Agriculture. Values for typical Portuguese foods were added, based on the Portuguese tables of food composition, typical recipes and data from previous studies[25]. The nutrient content of food items which are usually eaten cooked was estimated by considering cooking and processing.

Assessment of other covariates

Questionnaires

Adolescent smoking status was self-reported and participants were classified as never or ever smokers, this last category comprising those that just experienced a puff up to currently smoking adolescents.

Drinking behaviour was assessed through the question "Have you ever drunk an alcoholic beverage?". Adolescents were classified as "never drinker" or "ever drinker", this last category comprising those who had only experimented to those who drink at least once per month.

Physical exercise was assessed using a questionnaire covering various daily activities. Sport activities were considered as regular scheduled sport activities outside school.

Leisure time activities were classified according to a multiple-choice question proposing four subjective intensity categories (mainly sitting, mainly standing, active or very active). Average weekend time spent watching TV, playing computer and reading was recorded and added to compute total time spent in sedentary activities.

Parental education level was measured as the number of successfully completed years of formal schooling and adolescents were classified according to the parent with the higher education level.

Family history of disease comprised information on diabetes, hypertension and dyslipidaemia. It was asked if the disease had ever been diagnosed, separately for

each disease and for each parent. The possible answers were "yes", "no" and "do not know".

Anthropometrics

Weight and height were obtained with the subject in light indoor clothes and no shoes. Weight was measured in kilograms, to the nearest tenth, using a digital scale and height was measured in centimetres, to the nearest tenth, using a portable stadiometer. Adolescents were classified according to the age- and sex-specific BMI based on the Center for Disease Control and Prevention's 2000 BMI-for-age growth charts for the United States[26]. Based on this data we classified those above the 95th percentile as obese and those between the 85th and 95th percentiles as overweight. Body fat percentage was estimated by bioelectrical impedance, using Tanita® (Tanita TBF-300, Tanita Corporation of America, Inc., Illinois, USA).
Waist circumference was measured to the nearest centimetre, midway between the lower limit of the rib cage and the iliac crest, with the subject standing, using a flexible and non-distensible tape and avoiding exertion of pressure on the tissues.

Blood pressure

Blood pressure was measured with a mercury sphygmomanometer using the auscultatory method, following the recommendations of the American Academy of Paediatrics[27]. Classification was based on the mean of two readings taken on a single occasion, separated by at least 10 minutes' rest, with a third reading being taken if the difference between the first two was over 5 mmHg. High blood pressure was defined according to the quantitative criteria of the American Academy of Paediatrics[27]: systolic or diastolic blood pressure above the 90th percentile for age, gender, and height.

Laboratory measures

A venous blood sample was drawn after a 12-hour overnight fast. All the samples were analysed at the central laboratory of the university hospital. Serum glucose, cholesterol and triglycerides were determined using automatic standard routine enzymatic methods. High-density lipoprotein (HDL)-cholesterol was determined after precipitation of apolipoprotein B-containing lipoproteins. Serum insulin was measured using a 125I-labelled insulin radioimmunoassay method, and insulin resistance was

estimated according to the homeostatic model assessment (HOMA), as the product of fasting glucose (mmol/L) and insulin (μUI/mL) divided by a constant 22.5[28].

Metabolic syndrome

For this analysis metabolic syndrome components were defined according to the National Cholesterol Education Program Adult Treatment Panel III definition modified for age[29].

Components were defined as follows: (1) waist circumference ≥ 75th percentile[30] for age and gender; (2) HDL cholesterol ≤ 50 mg/dL; (3) triglycerides ≥ 110 mg/dL; (4) systolic or diastolic blood pressure ≥ 90th percentile for age, gender, and height[31]; and (5) fasting glucose ≥ 100 mg/dL. The presence of the metabolic syndrome phenotype was defined as having 3 or more of these 5 characteristics[29].

Participants

Two thousand seven hundred and eighty-six eligible participants were identified. Among them, forty-four (1.6 %) could not be reached, 583 (20.9 %) were refusals (no signed informed consent form was returned) and 2159 agreed to participate and provided information at least for part of the planned assessment, resulting in an overall participation rate of 77.5 %, similar in public (77.7 %) and private (77.0 %) schools (p=0.709).

Of the 2159 participants, 247 did not return the home questionnaire and 297 did not fill in the FFQ or were excluded because no information was provided on more than 10 % of food items. A further ninety-three participants were not considered for the current analysis because their total energy intake was more than 3 times the interquartile range or their intake of fruit or vegetables was more than 1.5 times the interquartile range. Additionally, 72 participants did not perform the anthropometric measurements; other 409 did not undergo blood collection, as well as 8 participants did not perform blood pressure measurement. Thus, the analysis was based on the information of 1033 participants.

Statistical Analysis

The distribution of quantitative variables was checked using the Kolmogorov-Smirnov test. Proportions were compared using the chi-square test or Fisher's exact test,

whenever appropriate, and quantitative variables using the Kruskal-Wallis or Mann-Whitney test, the results being presented as medians (percentile 25 - percentile 75).
Crude and adjusted means and 95% confidence intervals (95% CI) of selected anthropometric and metabolic characteristics were calculated according to quartiles of vitamin D intake, using liner regression adjusted for gender, BMI, physical activity and trimester of evaluation.
Logistic regression models were used to estimate odds ratio and 95% CIs for metabolic syndrome and its components according to quartiles of vitamin D intake. Models were adjusted for gender, BMI, physical activity and trimester of evaluation.
In all tests, statistical significance was considered with an alpha critical value of 0.05.
Statistical analyses were performed using the Statistical Package for the Social Sciences (IBM® SPSS® Statistics), version 21.0.

Ethical Considerations

The Ethics Committee of Hospital S. João approved the study and written consent was obtained from both legal guardians and adolescents. In accordance to the study protocol, the results of the assessments were provided as individual reports addressed personally to the adolescents' legal guardians, with an explicit recommendation that they should be communicated to the family physician. This indication was given in all cases, irrespective of the results.

Results

In this analysis we included 1033 participants and their characteristics were compared to those from excluded adolescents (table 1). Both groups were similar, except for the type of school, with participants not included being mostly from public schools, and with less educated parents, presenting higher activity spent in leisure time outside school and less alcohol consumption.
Table 2 presents the description of metabolic syndrome and its components in our sample. The most prevalent component was low HDL cholesterol, with 53.3% of participants showing values equal to or below 50 mg/dL. In contrast, the component with the lowest prevalence was high glucose levels (0.6%). We found a prevalence of metabolic syndrome of 13.2%.

Table 3 displays the median of vitamin D intake according to demographic and clinical characteristics of the participants. We observed a very low vitamin D intake of 4.11 (2.88-5.60) µg among participants. The median of vitamin D intake was higher among adolescents who reported the use of vitamin supplements and, although not statistically significant, there was a trend toward a lower mean vitamin D intake in girls and in those who practice less physical activity. The intake was lower in those defined as obese or abdominally obese, as well as in those who had low HDL cholesterol. No differences were observed in vitamin D intake by blood pressure, level of glucose, level of triglycerides and the metabolic syndrome.

The crude mean (95% CI) of selected anthropometric and metabolic characteristics according to quartiles of vitamin D intake is shown in table 4. Significant trends for lower BMI and waist circumference were observed in adolescents with higher vitamin D levels, although the latter not showing statistical significance. No association was observed between vitamin D intake and the other components evaluated. After adjusting for gender, BMI physical activity, and trimester of evaluation no significant difference was observed (table5).

The multivariable-adjusted associations between vitamin D intake and metabolic syndrome and their components are shown in Table 6. Even though no significant association was observed, the adjusted odds ratios for BMI and HDL cholesterol increased with the decreasing quartile of vitamin D intake. It was not computed the Odds Ratio regarding elevated fasting glucose, due to the very low prevalence of this component.

Discussion

In this sample of 13-year-old adolescents we found a very low vitamin D intake from diet. The median of vitamin D intake was 4.11 (2.88-5.60) µg, far below the 10 µg recommended by IOM, reinforcing the descriptions of the worldwide high prevalence of vitamin D deficiency [32, 33], also observed in adolescents from other European countries [34].

Despite the low intake, a mild increase of prevalence of obesity and central obesity was observed with the decreasing quartile of vitamin D, however the effect was not statistically significant. Few studies have addressed the effect of dietary vitamin D on cardiovascular risk factors [13, 35, 36], and to best of our knowledge none has addressed the period of adolescence.

Once the human body through the action of sunlight can synthesize vitamin D and serum concentrations were not determined in the current analysis, we cannot fully

confirm this hypothesis. However, although 25(OH)D concentration in the human body is the biologically internal dose of the total exposure of vitamin D, this work strengthens the need to take into consideration the part of vitamin D that comes from diet and supplementation, since in a country plentiful in sun, it is observed an inverse relation between some of the most prevalent components of the metabolic syndrome and vitamin D intake.

Trends for a lower BMI were verified in adolescents with higher vitamin D levels. Although it has often been suggested the sequestration within adipose tissue as an explanation for the lower levels of vitamin D in subjects with a higher BMI [37, 38], our results indicate that the lower vitamin D intake by these individuals should also be considered as a possible explanation.

Although there is increasing evidence that vitamin D may be a negative endocrine regulator of the renin-angiotensin system, through the inhibition of the renin gene expression by the activated metabolite of 25(OH)D, 1,25-dihydroxyvitamin D (1,25$[OH]_2$D), in the current study, we observed no independent cross-sectional association among dietary vitamin D, systolic and diastolic blood pressure, and hypertension. These findings are in agreement with a large prospective study of Forman et al. in 2005 that found no association between vitamin D intake from diet and supplements and the risk of incident hypertension[39]. No relation was also observed between triglycerides across increasing quartiles of vitamin D intake, which is accordant with Fung et al., who evaluated associations of dietary and supplemental vitamin D intake with the 20-y incidence of metabolic syndrome from 4727 black and white young men and women from the Coronary Artery Risk Development in Young Adults study (CARDIA) and found no relation between blood pressure and triglycerides across increasing quintiles of vitamin D[13].

No differences were observed between quartiles of vitamin D and glucose, insulin and, consequently, neither in HOMA. A possible reason for this observation is that the majority of participants are considered healthy and the measures to assess insulin resistance may not be the most appropriate. The gold standard assessment of insulin resistance requires an invasive and impractical application in children and adolescents [40], therefore, simple tools were considered valid, as fasting glucose, insulin levels and the HOMA index[41-43].

Despite the extremely low intake of vitamin D, it was observed an inverse relation with high BMI and low HDL cholesterol. Although some studies found an inverse association between vitamin D and dyslipidaemia, is not yet clarified the mechanism that can explain this observation [44].

This investigation had strengths and limitations. The FFQ has some restraints in assessing dietary intake, which include the use of a predetermined food list that might not be representative of foods eaten by a specific population [23]. However, we believe that this possible bias had a very low effect, because this FFQ was validated for the adult population [25] and some foods or food groups eaten more frequently by the adolescent age group were included in the questionnaire. Likewise, adolescents were encouraged to list foods eaten at least once weekly not enumerated in the FFQ, in an open section. The reliance on participants' recall and the requirement of motivated participants, especially in self-administered questionnaires is another limitation of the FFQ[23]. Although it was self-administered, adolescents were given oral instructions on filling it in and written instructions were also sent home along with the questionnaire. Despite having completed it with the help of their parents or legal guardians, which may have improved the quality of information, the extent to which parents might have an influence on overestimating healthy foods (according to what is socially acceptable) and on underestimating unhealthy foods is unknown.

We chose not to evaluate data regarding the portion size of the food intake. We expect this option to not have an impact on our results since previous studies suggest that to record information on portion size does not substantially improve the assessment of dietary intake[23], which may be more relevant in adolescents who may have difficulty in estimating the portions and frequently ignore these questions when questionnaires are self-administered[45].

A potentially important source of vitamin D originates from sun exposure, which was not measured in this study. Since sunlight exposure substantially affects vitamin D levels, this might represent a limitation, as data on sun exposure were not available in EPITeen datasets. Nonetheless, the analysis was conducted over the year and adjusted for trimester of evaluation to take into account seasonal variation. Information was collected on the type of supplement taken, allowing to understand whether participants were taking vitamin D supplementation, being confirmed the high dose of vitamin D for the most part, whereas it was not possible to confirm the composition for a minority. Thus, it was considered whether the participant took or did not take the supplement, assuming that those who took were taking high doses of vitamin D. Once this information did not change the results, the variable was not considered in the final models.

Notwithstanding the above limitations, this study is one of the few that has assessed vitamin D intake in adolescents and its relation with the metabolic syndrome. Moreover, the dataset employed was from a population-based sample, which may enhance generalizability. However, because data are cross-sectional, the causative

nature of associations cannot be determined. Yet, it is no expected that participants have altered their intake in consequence of the components evaluated. Therefore, we can assume that the relation verified is not the result of inverse causality. Future studies with a long-term longitudinal design are warranted to better understand the mechanisms that can explain the relation observed.

Conclusion

In summary, despite the extremely low vitamin D intake, an inverse relation with high BMI and low HDL cholesterol was observed. Even with an intake lower than recommended, there is a decreasing *odds* of this components with the increasing intake of vitamin D, though without statistical significance. Additional research is necessary to determine the long-term consequences of vitamin D deficiency, including whether low vitamin D levels during childhood significantly predict the occurrence of cardiovascular disease in adulthood.

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Table 1. Description and comparison of participants' characteristics included and excluded in the analysis: 13-year-old adolescents, Porto, Portugal, 2003/2004 school year.

	Excluded (n=1126)		Included (n=1033)		
Characteristic	n	%	n	%	*p*
Sex					
Girls	562	49.9	554	53.6	0.084
Boys	564	50.1	479	46.4	
School					
Public	896	79.6	754	73.0	<0.001
Private	230	20.4	279	27.0	
Parental education (years)					
0-6	327	31.4	240	23.5	0.001
7-9	203	19.5	218	21.3	
10-12	255	24.5	281	27.5	
>12	256	24.6	284	27.8	
Missing (n)	85		10		
Leisure-time physical activity					
Mainly sitting	228	26.6	281	27.2	0.014
Mainly standing	161	18.8	234	22.7	
Active	224	26.1	306	29.6	
Very active	176	20.5	164	15.9	
Missing (n)	68		48		
Fruit consumption (portions/week)					
<1/day	397	35.3	457	44.2	0.185
≥1 and < 2/day	265	23.5	370	35.8	
≥2/day	147	13.1	188	18.2	
Missing	317		18		
Vitamin supplementation					
No	638	72.7	779	75.4	0.483
Yes	182	20.8	205	19.8	
Missing	306		49		
Smoking					
No	757	74.8	769	75.2	0.796
Yes	227	22.4	237	23.2	
Missing	142			27	
Alcohol intake					
No	484	43.0	441	42.7	0.024
Yes	506	44.9	565	54.7	
Missing	136		27		
Family history of disease					
Diabetes					
No	816	72.5	895	86.6	0.120
Yes	79	7.0	76	7.4	
Do not know	33	2.9	21	2.0	
Missing	198		41		
Cholesterol					
No	568	50.4	604	58.5	0.004
Yes	318	28.2	374	36.2	
Do not know	62	5.5	35	3.4	
Missing	178		20		
Hypertension					
No	678	60.2	731	70.8	0.101
Yes	235	20.9	261	25.3	
Do not know	36	3.2	22	2.1	
Missing	177		19		
Anthropometrics					
BMI <85th	767	76.3	758	73.4	0.306
BMI ≥85th and <95th	149	14.8	170	16.5	
BMI ≥95th percentile	88	8.9	105	10.2	
Missing	121		0		

Table 2. Characteristics of the participants according to metabolic syndrome and its components.

Characteristic	Median (P25-P75) or %
Waist circumference ≥75th percentile	26.0
Blood pressure	
Systolic blood pressure, mm Hg	112.0 (105.0-1220.0)
Diastolic blood pressure, mm Hg	68.0 (62.0-73.0)
High blood pressure[a]	33.6
Diabetes related measures	
Fasting glucose, mg/dL	85.0 (80.0-91.0)
Fasting glucose ≥ 110mg/dL	0.6
Lipids	
HDL cholesterol ≤ 50 mg/dL	53.3
HDL cholesterol, mg/dL	48.0 (42.0-56.0)
Triglycerides ≥ 100 mg/dL	11.5
Triglycerides, mg/dL	58.0 (46.0-78.0)
Metabolic syndrome[b]	13.2

[a] Data are defined as systolic or diastolic blood pressure ≥ 90th percentile for age, gender and height[(27)]

[b] Data are defined according to the method of de Ferranti et al[(29)]

Table 3. Vitamin D intake (μg) according to participants' characteristics.

Variable	n	Vit D intake Median (P25-P75), μg	*P*
Overall	1033	4.11 (2.88-5.60)	
Sex			
Girls	554	4.02 (2.80-5.43)	0.050
Boys	479	4.26 (2.99-5.72)	
Vitamin Supplementation			
No	779	4.02 (2.86-5.48)	0.014
Yes	205	4.42 (3.02-6.12)	
Parental education (years)			
0-6		4.13 (2.98-5.67)	0.462
7-9		4.30 (2.82-5.86)	
10-12		4.27 (2.30-5.35)	
>12		3.85 (2.76-5.47)	
Physical activity			
Mainly sitting	281	3.94 (2.71-5.55)	0.062
Mainly standing	234	3.97 (2.78-5.55)	
Active	306	4.15 (3.00-5.44)	
Very active	164	4.47 (3.01-6.14)	
BMI ≥95th percentile[a]			
No	928	4.16 (2,96-5.63)	0,005
Yes	105	3.73 (2.43-5.08)	
Waist circumference ≥75th percentile[a]			
No	764	4.16 (2.95-5.68)	0,005
Yes	269	3.87 (2.72-5.06)	
High blood pressure[b]			
No	686	4.13 (2.91-5.94)	0,763
Yes	347	4.06 (2.83-5.63)	
Fasting glucose ≥ 110mg/dL			
No	1027	4.11 (2.89-5.60)	0,690
Yes	6	3.80 (2.65-5.28)	
HDL cholesterol ≤ 50 mg/dL			
No	482	4.28 (3.05-5.63)	0,004
Yes	551	3.90 (2.75-5.50)	
Triglycerides ≥ 100mg/dL			
No	914	4.07 (2.89-5.53)	0,218
Yes	119	4.37 (2.84-6.01)	
Metabolic Syndrome[c]			
No	897	4.15 (2.94-5.58)	0,209
Yes	136	3.85 (2.65-5.67)	

[a] Data are according to age and gender.
[b] Defined as systolic or diastolic blood pressure above the 90th percentile for age, gender, and height[(27)]
[c] Defined according to the definition of *de Ferranti et al*.[(29)]

Table 4. Mean (95% CI) of selected anthropometric and metabolic characteristics according to quartiles of vitamin D intake.

Variable	Quartiles of Vitamin D intake, µg				*P*
	I (<2.88)	II (2.88-4.10)	III (4.11-5.60)	IV (>5.60)	
Blood Pressure, mm Hg					
Systolic	113.3 (111.9-114.8)	113.8 (112.4-115.2)	112.1 (110.8-113.4)	112.6 (111.2-114.0)	0,301
Diastolic	67.6 (66.6-68.6)	67.6 (66.6-68.5)	68.0 (66.9-69.1)	68.3 (67.4-69.3)	0,680
Lipids, mg/dL					
Total cholesterol	166.7 (162.8-170.6)	165.5 (161.7-169.3)	166.9 (163.0-170.8)	165.7 (161.9-169.4)	0.940
HDL cholesterol	48.1 (46.7-49.5)	48.9 (47.6-50.2)	50.6 (49.2-52.0)	49.1 (47.6-50.6)	0.088
LDL cholesterol	105.3 (102.1- 108.5)	103.7 (100.6- 106.7)	103.6 (100.6- 106.6)	103.4 (100.4- 106.4)	0.827
Triglycerides	66.5 (63.0- 70.0)	64.7 (60.9- 68.5)	63.4 (60.0- 66.9)	65.9 (62.4- 69.4)	0.639
Diabetes-related measures					
Fasting glucose, mg/dL	84.2 (83.0-85.3)	84.6 (83.5-85.8)	85.3 (84.1-86.5)	86.1 (84.9-87.3)	0.113
Fasting insulin, µU/mL	7.9 (7.2-8.7)	8.1 (7.3-9.0)	8.1 (7.4-8.8)	7.7 (7.0-8.4)	0.867
Homa -IR	1.7 (1.5-1.8)	1.7 (1.5-1.9)	1.7(1.6-1.9)	1.7 (1.5-1.8)	0.926
Anthropometrics					
BMI	21.6 (21.1-22.1)	21.2 (20.8-21.6)	20.9 (20.5-21.3)	20.7 (20.3-21.1)	0.023
Waist circumference	73.5 (72.5-74.6)	72.9 (71.8-74.0)	72.8 (71.7-73.9)	71.6 (70.7-72.6)	0.088

Table 5. Adjusted mean (95%CI) of selected anthropometric and metabolic characteristics according to quartiles of vitamin D intake among participants.

Variable	Quartiles of Vitamin D intake, µg				*P*
	I (<2.88)	II (2.88-4.10)	III (4.11-5.60)	IV (>5.60)	
Blood Pressure, mm Hg					
Systolic[a]	117.0 (115.4-118.6)	117.7 (116.1-119.3)	116.1 (114.4-117.7)	116.6 (115.0-118.2)	0.380
Diastolic[a]	70.1 (68.9-71.3)	70.2 (69.0-71.4)	70.9 (69.7-72.1)	71.1 (69.9-72.3)	0.411
Lipids, mg/dL					
Total cholesterol[a]	167.1 (162.4-171.7)	165.7 (161.0-170.4)	166.4 (161.7-171.2)	165.6 (161.0-171.4)	0.945
HDL cholesterol[a]	47.0 (45.3-48.6)	47.2 (45.5-48.8)	48.2 (46.5-49.8)	46.7 (45.1-48.3)	0.435
LDL cholesterol[a]	105.8 (102.0- 116.2)	104.5 (100.7-108.3)	104.2 (100.4- 108.0)	104.5 (100.8-108.2)	0.906
Triglycerides[a]	71.3 (67.5-76.0)	70.1 (65.8-74.4)	70.2 (65.9-74.5)	72.0 (67.8-76.2)	0.822
Diabetes-related measures					
Fasting glucose, mg/dL[a]	84.8 (83.4-86.2)	85.6 (84.2-87.1)	86.4 (84.9-87.8)	86.7 (85.2-88.1)	0.139
Fasting insulin, µU/mL[a]	9.6 (8.8-10.5)	10.2 (9.3-11.1)	10.4 (9.5-11.3)	9.8 (9.0-10.7)	0.529
Homa -IR[a]	2.0 (1.8-2.2)	2.2 (2.0-2.4)	2.2(2.0-2.4)	2.1 (1.9-2.3)	0.383
Anthropometrics					
BMI[b]	21.2 (20.7-21.7)	20.9 (20.5-21.4)	20.7 (20.2-21.2)	20.5 (20.1-21.0)	0.142
Waist circumference [a]	79.0 (78.3-79.8)	79.6 (78.8-80.3)	79.6 (78.8-80.4)	78.7 (78.0-79.5)	0.180

[a] Adjusted for gender, BMI, physical activity and trimester of evaluation.

[b] Adjusted for gender, physical activity and trimester of evaluation.

Table 6. Adjusted odds ratios and 95% CIs of metabolic syndrome and its components according to vitamin D intake among participants.

Variable	I (<2.88)	II (2.88-4.10)	III (4.11-5.60)	IV (>5.60)
BMI ≥ 95th percentile[a]				
Prevalence, %	15.2	8.9	8.1	8.5
Adjusted OR (95% CI)	1.78 (1.00-3.17)	1.00 (0.53-1.88)	0.93 (0.49-1.78)	1.00 (reference)
Waist circumference ≥ 75th percentile[b]				
Prevalence, %	30.0	27.5	25.5	21.2
Adjusted OR (95% CI)[b]	1.01 (0.50-2.05)	1.29 (0.65-2.57)	1.27 (0.64-2.50)	1.00 (reference)
High blood pressure[c]				
Prevalence, %	35.0	32.9	32.8	33.6
Adjusted OR (95% CI)[b]	0.99 (0.67-1.49)	0.95 (0.64-1.41)	0.98 (0.66-1.45)	1.00 (reference)
Fasting glucose ≥ 110mg/dL[†]				
Prevalence, %	0.8	0.4	0.8	0.4
HDL cholesterol ≤ 50 mg/dL				
Prevalence, %	59.1	55.0	47.9	51.4
Adjusted OR (95% CI)[b]	1.22 (0.84-1.78)	1.07 (0.76-1.58)	0.86 (0.60-1.25)	1.00 (reference)
Triglycerides ≥ 110 mg/dL				
Prevalence, %	12.1	8.9	10.4	14,7
Adjusted OR (95% CI)[b]	0.76 (0.44-1.32)	0.50 (0.28-0.90)	0.70 (0.40-1.22)	1.00 (reference)
Metabolic syndrome[d]				
Prevalence, %	16.0	12.4	10.8	13.5
Adjusted OR (95% CI)[b]	0.77 (0.41-1.46)	0.64 (0.34-1.24)	0.58 (0.29-1.15)	1.00 (reference)

[a] Adjusted for physical activity and trimester of evaluation; BMI classified according to age and gender[(29)]

[b] Adjusted for gender, physical activity, BMI and trimester of evaluation.

[c] Defined as systolic or diastolic blood pressure above the 90th percentile for age, gender, and height[(27)].

[d] Defined according to the definition of de Ferranti et al[(29)].

[†] Few cases to measure the association

CONCLUSIONS

This research, based on a population-based sample of Portuguese adolescents, allowed the following conclusions:

A very low intake of vitamin D was observed, with a median (percentile 25-percentile 75) of 4.11 (2.88-5.60) μg, far below the 10 μg recommended by IOM, supporting the possibility of having a high prevalence of vitamin D deficiency.

A highly prevalence of metabolic syndrome was found (13.2%). Regarding its components, the most prevalent were high blood pressure and dyslipidaemia. Notwithstanding the extremely low vitamin D intake, an inverse relation with high BMI and low HDL cholesterol was observed, though without statistical significance. No trend was found for metabolic syndrome.

These results reinforce the need for preventive measures in order to revert, or minimize, the risk of cardiovascular disease later in life. One of these measures may be to improve the vitamin D intake, which, beyond the cardiovascular protective effect, also could promote other health benefits.

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