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Low caloric intake, from adolescence into adulthood: effect on insulin sensitivity and cardiometabolic risk factors

Joana Pinto da Costa

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Low caloric intake, from adolescence into adulthood: effect on insulin sensitivity and cardiometabolic risk factors

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Abbreviations

AL	*Ad Libitum*
BMI	Body Mass Index
BMR	Basal Metabolic Rate
BP	Blood Pressure
CI	Confidence Interval
CR	Caloric Restriction
CVD	Cardiovascular disease(s)
EI	Energy Intake
EPITeen	Epidemiological Health Investigation of Teenagers in Porto
estBMR	Estimated Basal Metabolic Rate
FFQ	Food Frequency Questionnaire
FPG	Fasting Plasma Glucose
GD	Gestational Diabetes
HDL-C	High Density Lipoprotein-Cholesterol
HOMA-IR	Homeostatic Model Assessment for Insulin Resistance
IDF	International Diabetes Federation
IFG	Impaired Fasting Glucose
IGT	Impaired Glucose Tolerance
IR	Insulin Resistance
Kcal	kilocalorie(s)
kg/m^2	kilograms *per* meter square
LDL-C	Low Density Lipoprotein-Cholesterol
MetS	Metabolic Syndrome
NCD	Non-Communicable Disease(s)
NCEP ATP III	National Cholesterol Education Program - Adult Treatment Panel III
NHLBI	National Heart, Lung, and Blood Institute
OGTT	Oral Glucose Tolerance Test
PAL	Physical activity level
repEI	Reported Energy Intake
SD	Standard Deviation
SES	Socioeconomic status
T1D	Type 1 Diabetes

T2D	Type 2 Diabetes
TG	Triglyceride
WC	Waist Circunference
WHO	World Health Organization

Abstract

Introduction: Caloric restriction has been studied as it seems to increase lifespan and delay diseases onset in rodents and short-lived species. Although the effects in primates are years away from being well established, caloric restriction has also been associated with improved cardiometabolic risk factors, for both non-human and human primates. However, available data is based on studies with too short period of follow-up and very restrictive diets. We aimed to evaluate, in a longitudinal study, how the adherence to a dietary pattern characterized by a lower caloric intake at 13 years old affects cardiometabolic risk factors on young adulthood (21 years old).

Methods: The study was based on data from participants on the EPITeen cohort - Epidemiological Health Investigation of Teenagers in Porto -, with valid dietary information at 13y (n=962) and at 21y (n=607). At both waves diet was evaluated by a food frequency questionnaire, body mass index, blood pressure, insulin, triglycerides, and glucose were also assessed at both waves. Metabolic syndrome features were defined according to the National Cholesterol Education Program Adult Treatment Panel III definition and an adaption was used at 13 years old. The Homeostatic Model Assessment for Insulin Resistance was used to assess insulin resistance. Four dietary patterns were previously identified at 13y: *Healthier* (n=239; 16.1%), *Dairy Products* (n=442; 29.7%), *Fast food & Sweets* (n=212; 14.2%) and *Lower Intake* (n=596; 40%). The dietary patterns found at 13y seems to be predictor of the dietary pattern found at 21y, although the differences between patterns are more tenue.

Results: The mean daily energy intake considering all the participants was 2394.4 Kcal/d at 13 years old and 2279.6 Kcal/d at 21 years old. The *Lower Intake* dietary pattern has the lowest mean energy intake at both ages, 1806.5 Kcal/d and 2180.8 Kcal/d at 13 and 21 years old, respectively. The "Lower intake" pattern presented the highest proportion of overweight/obese participants (34.8% and 26.1%, at 13 and 21y, respectively) and the "Fast food & Sweets" the lowest (19.5% and 15.4% at 13 and 21y, respectively). In the cross-sectional analysis, adolescents belonging to the *Lower Intake* dietary pattern presented the lower values of glucose and insulin, triglycerides, and blood pressure, but the differences were only statistically significant for glucose and systolic blood pressure, when considering only the plausible reporters. No significant effect was found in the longitudinal approach.

Conclusion: Our data supports that a dietary pattern characterized by a lower energy intake may contribute to a better cardiometabolic profile, by promoting better glucose metabolism parameters and lower systolic blood pressure. These results become clearer after excluding the potential misreporters.

Keywords: adolescents; caloric restriction; cardiometabolic risk factors; cohort; energy intake; energy intake misreport; young adults

Resumo

Introdução: A restrição calórica tem sido estudada desde que foi, primeiramente, associada à capacidade de aumentar a longevidade e adiar o aparecimento das doenças em roedores e espécies com tempo de vida reduzido. Apesar dos efeitos em primatas ainda não estarem bem estabelecidos, a restrição calórica tem sido associada a melhorias nos fatores de risco cardiometabólico em primatas humanos e não humanos. No entanto, estes dados são baseados em estudos com períodos de seguimento reduzidos e dietas muito restritivas. Com este estudo longitudinal, pretendíamos avaliar de que forma a aderência a um padrão alimentar caracterizado por uma reduzida ingestão calórica aos 13 anos, poderia afetar os fatores de risco cardiometabólico no início da idade adulta (21 anos de idade).

Métodos: O estudo tem por base dados de participantes da coorte EPITeen - *Epidemiological Health Investigation of Teenagers in* Porto -, que tinham informação válida sobre a dieta aos 13 (n = 962) e aos 21 anos (n = 607). Nos dois momentos, a dieta foi avaliada por questionário de frequência alimentar. Também o índice de massa corporal, pressão arterial, triglicerídeos e glicose foram avaliados nos dois momentos. A síndrome metabólica foi definida de acordo com a definição do *National Cholesterol Education Program Adult Treatment Panel III* e uma adaptação foi usada aos 13 anos. O *Homeostatic Model Assessment for Insulin Resistance* foi usado para avaliar a resistência à insulina. Quatro padrões alimentares foram previamente definidos, aos 13 anos: *Healthier* (n=239), *Dairy products* (n=442), *Fast food & Sweets* (n=212) e *Lower Intake* (n=596). Estudos anteriores demonstraram que estes padrões aparentam predizer os padrões alimentares encontrados aos 21, apesar das diferenças entre padrões estarem mais atenuadas.

Resultados: A ingestão energética média diária, considerando todos os participantes, é de 2394.4 Kcal/d aos 13 anos e 2279.6 Kcal/d aos 21 anos. O padrão alimentar *Lower Intake* tem a ingestão calórica média mais baixa, 1806.5 Kcal/d e 2180.8 Kcal/d, aos 13 e aos 21 anos, respetivamente. O padrão alimentar *Lower Intake* apresentou a maior proporção de participantes com excesso de peso/obesos (34.8% e 26.1%, aos 13 e aos 21 anos, respetivamente). Na análise transversal, os adolescentes identificados no padrão alimentar *Lower Intake* apresentaram os valores mais baixos de glicose, insulina, triglicerídeos e pressão arterial. No entanto, as diferenças mostraram-se estatisticamente significativas apenas para a glicose e pressão arterial sistólica, quando considerados

apenas os participantes com declaração plausível da ingestão energética. Na análise longitudinal, não foram encontrados efeitos significativos.

Conclusão: Os nossos resultados sustentam que um padrão alimentar caracterizado por uma menor ingestão calórica pode contribuir para um melhor perfil cardiometabólico, promovendo melhores parâmetros do metabolismo da glicose e pressão arterial sistólica mais baixa. Estes resultados são ainda mais claros depois de excluir os participantes com declaração não plausível da ingestão energética.

Palavras-chave: adolescentes; coorte; fatores de risco cardiometabólico; ingestão energética; jovens adultos; plausibilidade da declaração energética; restrição calórica

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1. Introduction

1.1. Diabetes, Obesity and Metabolic Syndrome

Insulin is an anabolic peptide hormone produced by cells in the islets of Langerhans of the pancreas (1). Mainly associated with the regulation of the blood glucose levels, insulin is a major hormone in the energy metabolism, together with glucagon that has opposite action (1). In a healthy individual, pancreas releases insulin in response to increased plasma glucose levels (the most important insulin stimulus) – postprandial –, lowering the glucose level to a normal range, as it promotes the blood glucose uptake, and the synthesis of glycogen, protein and triacylglycerol, mainly in liver, muscle and adipocytes - the cells with higher sensitivity to insulin (1). In a pathological situation - insulin resistance - these cells do not respond properly to the normal concentration of circulating insulin (1). Triggering a compensatory process where the pancreatic beta cells will produce more insulin than usual – hypersecretion of insulin, leading to hyperinsulinemia (higher than normal insulin concentration levels). While the insulin produced is enough to overcome the weak cell response to insulin, the blood glucose levels will stay in a healthy range – euglycemia -, but eventually, beta cell become dysfunctional - pancreas will fail to secrete enough insulin to compensate the insulin resistance (1, 2). This combination of insulin resistance and impaired insulin release, leads to a state of hyperglycaemia – the glucose levels higher than the normal range: impaired glucose tolerance, impaired fast glucose and may result in type 2 diabetes (1-3). Diabetes (or Diabetes Mellitus) is a heterogeneous group of syndromes, a chronic condition, primarily characterized by an elevation of the blood glucose levels (1, 4).

There are three major types of diabetes: type 1 diabetes (T1D), type 2 diabetes (T2D) and gestational diabetes (GD) (4). T1D or insulin-dependent, juvenile, or childhood-onset diabetes, as it usually is diagnosed during childhood or adolescence and requires insulin administration. It is characterized by a deficient or no insulin production caused mostly by an auto-immune response, where the body attacks the insulin-producing beta cells. Characterized by elevated blood glucose level, and the presence of polyuria (excessive excretion of urine), polydipsia (excessive thirst) and/or polyphagia (constant hunger), accompanied by fatigue, weight loss and weakness. The causes are not fully understood, but has been associated to genetic susceptibility and external triggers, such as diet, viral infection or toxins, however, at the actual knowledge does not derive from preventable behaviours (1, 4, 5). T2D or non-insulin-dependent, despite being preventable, is the most prevalent type of diabetes (6), where thereby the pancreas does not produce enough

insulin combined with insulin resistance (1). Usually can be treated with oral medication but may also require insulin. Used to be known by adult-onset diabetes, once that for many years was seen only in adults, but currently, beyond the early onset in young adults is increasingly diagnosed in children and adolescents (7). Rising parallelly as the number of overweight and obese individuals increases, as obesity is considered a strong risk factor to T2D (4, 5, 7). Gestational diabetes (GD) or hyperglycaemia in pregnancy is specific to pregnant women when the insulin is less effective than before, due to hormone production by placenta. GD increases the risk of complications during pregnancy and there is an associated long-term risk of T2D for the mother (4, 5, 7).

The diagnostic criteria of diabetes are based on plasma glucose estimation, summarized on Table 2.1., usually measured after fasting (generally an overnight fasting) or after ingesting a standard amount of glucose during an oral glucose tolerance test (OGTT), the only mean to identify subjects with impaired glucose tolerance (8).

Table 2.1. Diagnostic Criteria. Diabetes and Prediabetes (Impaired Glucose Tolerance; Impaired Fasting Glucose) Adapted from: IDF Diabetes Atlas Eighth edition 2017 (4).

Diabetes	IGT	IFG
FPG ≥7.0 mmol/L (126mg/dL) or 2-h after meal plasma glucose ≥11.1 mmol/L (200mg/dL). or **random** glucose >11.1 mmol/L (200 mg/dL) or HbA1c ≥ 48 mmol/mol (equivalent to 6.5%)	FPG <7.0 mmol/L (126mg/dL) and 2-h OGTT ≥ 7.8 and <11.1 mmol/L (140 and 200mg/dL)	FPG ≥ 6.1 and ≤6.9 mmol/L (110 mg/dL to 125 mg/dL) and (if measured) 2-h OGTT <7.8 mmol/L (140 mg/dL)

Abbreviations: FPG, Fasting Plasma Glucose; HbA1c, Glycated Haemoglobin; IGT, Impaired Glucose Tolerance; OGTT, Oral Glucose Tolerance Test; IFG, Impaired Fasting Glucose.

Glycated haemoglobin (HbA1C) measure reflects the average plasma glucose in the previous 2 to 3 months, mainly used to evaluate glycaemic control on people with diabetes, has been discussed as a diagnostic approach as it does not require any specific preparation (can be performed in nonfasting state) (7, 8). Impaired glucose tolerance (IGT) and impaired fasting glucose (IFG), also defined as prediabetes, are risk factors to T2D and the risk is greater when they are present concomitantly (4). Prediabetes is diagnosed when blood glucose is higher than normal, but below the diagnostic threshold for diabetes (4). World Health Organization (WHO) uses the term “Intermediate Hyperglycaemia” rather than prediabetes (8), a cornerstone to change lifestyle and convert back to euglycemia, reducing the risk to develop diabetes (9). Prediabetes

diagnose has been also associated with complications usually present in subjects with diabetes, such as damage on eyes, kidneys, blood vessels and the heart and also early forms of nephropathy and chronic kidney disease (9).

International Diabetes Federation (IDF) estimated that in 2017, there were 451 million adults (18-99 years) with diabetes worldwide (4). This number becomes even more worrying as it is estimated that 50% of the cases remains undiagnosed (4). It is expected that by 2045, 693 million adults (18-99 years) worldwide will have diabetes (10). In Europe, in 2017, diabetes prevalence was estimated to be 8.8%, representing 58.0 million people (20-79 years) with diabetes, and is expected to rise to 66.7 million by 2045 (4). In Portugal, in 2017, the estimated prevalence of diabetes among adults was estimated to be 13.9% (11).

Diabetes, both type 1 and type 2, is associated with reduced life expectancy (12, 13), higher rates of morbidity with an increased risk of develop disabling and life- threatening health problems (1, 4). WHO estimates that diabetes was directly the death cause of 1,6 million people worldwide in 2016, being among the top 10 global death causes (14). Due to lack of data on diabetes related mortality of many countries, obtaining this estimative is challenging. IDF estimates that in 2015 approximately 5 million people, between 20 and 79 years old died from diabetes (15). Nevertheless, although the prevalence is increasing it is expected that the mortality among diabetic individuals will decrease, in accordance with the Swedish study that compared mortality among diabetics, from 1998 to 2014 (16). The consistent elevation of blood glucose levels causes the chronic complications of diabetes: microvascular damage (retinopathy, nephropathy, and neuropathy) and macrovascular complications (cardiovascular disease, such as stroke and coronary artery disease) (1, 4, 15). Moreover, those with diabetes have up to threefold greater risk of developing CVD, the leading morbidity and mortality cause among individuals with T2D (4). Diabetes is a major cause of blindness, kidney failure and amputation (17). Controlling the blood glucose levels as normal as possible, as well as, blood pressure and cholesterol levels may prevent or delay the diabetes complications (15).

Beyond the direct mortality and morbidity burden, diabetes impacts significantly expenditures in health. For both countries and healthcare systems, individuals with diabetes and their families alike (4). The economic burden of diabetes is large, and it is

expected to keep increasing worldwide (18, 19). IDF estimates that by 2045, considering the group from 18 to 99 years old, the expenditure on diabetes will reach USD 958 billion (4).

Despite some of the risk factors for T2D are not modifiable, such as age, sex, ethnicity and family history, there are others that are, such as diet, physical inactivity, nutritional status and environmental exposures (4). Exercise regularly, have a healthy diet, avoid tobacco, achieve, and maintain a normal body weight may prevent or delay the onset of type 2 diabetes (17). Women with history of gestational diabetes or polycystic ovarian syndrome, older subjects, individuals with prediabetes and individuals that have familiars with type 2 diabetes are at higher risk of developing diabetes (3).

The causes of insulin resistance are not fully understood yet, however, it is known that insulin resistance increases with weight gain and decreases with weight loss (1), thus being overweight or obese are well-described risk factors that drive to an insulin resistance development, as insulin sensitivity is frequently reduced in obesity. Although, obesity and type 2 diabetes are associated with insulin resistance, most obese insulin resistant individuals will not develop hyperglycaemia, as long as, insulin secretion is enough to overcome the insulin resistance (3). Comparing with an individual with a normal weight, those who are overweight and obese have, respectively, approximately threefold risk and sevenfold risk of developing diabetes (20).

According to the WHO estimates, in 2016, more than 1.9 billion adults were overweight, 39% of the global adult population, of these, over 650 million were obese, 13% of the global adult population (21). The prevalence of obesity has nearly tripled between 1980 and 2016. Furthermore, overweight/obesity is no longer an adult pathology, 41 million children under the age of 5 were overweight/obese, in 2016, and this number is expected to increase. In addition, more than 340 million children and adolescents aged 5-19 years old were overweight/obese in the same period (21).

National Food, Nutrition, and Physical Activity Survey (IAN-AF), conducted from 2015 to 2016, estimates that almost 60% of Portuguese adults were overweight, from those approximately 35% were pre-obese and 22% were obese , according to the WHO criteria (22). The estimated prevalence of obese adults in Portugal, is higher than the WHO estimates of obesity prevalence in adults worldwide (13%) (21).

According the IAN-AF estimates, nearly 18% of children (<10 years) and 24% of adolescents (10 to 17 years) have pre-obesity, and around 8% of children and 9% of adolescents are obese (22). Portugal was, since the beginning of the COSI (Childhood Obesity Surveillance Initiative) study from WHO/Europe, one of the European countries with higher prevalence of overweight/obesity in childhood, although the prevalence appears to be decreasing, with a reduction of approximately 8% in the prevalence of overweight child, between 2008 and 2019 (23-25). The COSI Portugal 2019, estimates that 30% of the Portuguese children (aged 6 to 8 years) are overweight and 12% are obese (25). While the overall prevalence of overweight/obesity in children and adolescents (5 to 19 years old), in Europe, is 19% according HBSC (Health Behaviour in School-aged Children) study, in 2014 (26).

Halt the rise in obesity and diabetes, among others, is one of the measures of Global action plan for the prevention and control of noncommunicable diseases 2013-2020, a major public health challenge worldwide, with great socioeconomic impact (27) with 41 million of deaths worldwide attributed to non-communicable diseases annually (28). Cardiovascular diseases are the main contributors to mortality and morbidity among the non-communicable diseases and the leading death cause worldwide (29). The major risk factors to non-communicable diseases - such as, tobacco use, physical inactivity, unhealthy diet and the harmful use of alcohol (28) – are transversal to diabetes and obesity, and are associated with an increased risk of developing a combination of metabolic changes, frequently observed in simultaneous in the same individual - such as raised blood pressure, hyperglycaemia, and dyslipidaemia.

Reaven suggested in 1988 that insulin resistance/hyperinsulinemia may be a key factor on the development of these abnormalities and used the term Syndrome X to describe this cluster (30). This clustering of metabolic disturbances – interrelated risk factors to CVD and diabetes - have been labelled differently over the last century. Different organizations have been proposing several definitions to uniformize the criteria used worldwide (Table 1.3) (31-35). The Metabolic Syndrome (MetS) is the most worldwide accepted term and although the definition and diagnostic criteria are not consensual, the core components are common to the different definitions: obesity, insulin resistance, dyslipidaemia and hypertension (34, 35).

Table 1.3. Metabolic syndrome definitions and diagnostic criteria: WHO 1999 (31), NCEP ATP III 2001 (32, 34), IDF 2005 (34), IDF NHLBI 2009 (35).

	WHO 1999	**NCEP ATP III 2001**	**IDF 2005**	**IDF NHLBI 2009**
	IGT or diabetes and/or IR with two or more of the following:	**Three or more** of the following five risk factors:	Central obesity and **two or more** of the following:	**Three or more** of the following five risk factors:
Fasting Plasma Glucose		FPG ≥6.1 mmol/l (110 mg/dl)	FPG ≥ 5.6 mmol/l (100 mg/dl) or previously diagnosed T2D	FPG ≥5.6 mmol/l (100 mg/dl) or previously diagnosed T2D
Blood Pressure	Raised BP ≥140/90 mmHg	Raised arterial pressure ≥130/85 mmHg	Systolic BP ≥ 130 and/or diastolic ≥85 mmHg or on treatment for previously diagnosed hypertension	Systolic BP ≥ 130 and/or diastolic ≥85 mmHg or on treatment for previously diagnosed hypertension
Triglycerides	Raised plasma TG (≥1.7 mmol/l; 150 mg/dl) and/or low HDL-C (<0.9 mmol/l, 35 mg/dl men; <1.0 mmol/l, 39 mg/dl women)	Raised plasma TG (≥1.7 mmol/l; 150 mg/dl)	Raised plasma TG (≥1.7 mmol/l; 150 mg/dl)	Raised plasma TG (≥1.7 mmol/l; 150 mg/dl) or history of specific treatment for this lipid abnormality
HDL		<1.03 mmol/l, 40 mg/dl men; <1.29 mmol/l, 50 mg/dl women	HDL-C (<1.03 mmol/l, 40 mg/dl men; <1.29 mmol/l, 50 mg/dl women) or history of specific treatment for this lipid abnormality	HDL-C (<1.03 mmol/l, 40 mg/dl men; <1.29 mmol/l, 50 mg/dl women) or history of specific treatment for this lipid abnormality
Obesity	Waist-hip ratio (>0.9 men; >0.85 women) and/or BMI >30 kg/m2 Urinary albumin excretion rate ≥ 20 µg/min or albumin/creatinine ratio ≥30 mg /g	Waist circumference (>102 cm men, >88cm women)		Waist circumference (Population and country-specific definitions)

Abbreviations: WHO - World Health Organization; IDF - International Diabetes Federation; NCEP ATP III - National Cholesterol Education Program - Adult Treatment Panel III; AHA/NHLBI - American Heart Association/National Heart, Lung, And Blood Institute

Independently of the disagreements in the diagnostic criteria, primarily in cut-off values, it is clear from epidemiological data that the MetS is a frequent and increasing problem worldwide, and a significant predictor of incident diabetes, regardless of the definition used (36). Those with the syndrome have a twofold greater risk of CVD and a fivefold greater risk of developing type 2 diabetes and the risk is proportional to the number of MetS components verified (37).

It is estimated that approximately a quarter of the adult population worldwide has MetS (37). In Portugal this prevalence is even higher, PORMETS, a national cross-sectional study from February 2007 to July 2009, estimated a prevalence of the MetS varying from 37% to 50%, depending on the MetS definition used (38).

Concerning children and adolescents, there is a consensus regarding the existence of a similar clustering of those risk factors, however the discussion regarding the criteria to evaluate the MetS is even stronger (39). In a review including 27 studies 40 different definitions of MetS in paediatric age were used (39), most of them adaptations of the adult diagnose criteria such as NCEP-ATP III (32, 39, 40). IDF proposed, in 2007, a set of criteria's to diagnose MetS in children and adolescents, defending that it must be diagnosed only in children over 10 years (41, 42). Data from the Third National Health and Nutrition Examination Survey, 1988-1994 estimated a prevalence of 4% among the adolescent (12 – 19 years old), with a prevalence of almost 30% among the overweight adolescents (43). Similarly, a review, including studies from 2 to 19 years old estimated a prevalence ranging from 2% on normal weight children and adolescents to approximately 32% on obese (44). A review including 85 studies published between 2003 and 2011, estimated a prevalence of MetS around 3% in the whole population, but highly prevalent among obese children, estimated a mean prevalence of 29%, varying up to 66% (45). As the obesity has been increasing it is expected that the prevalence of MetS increases parallelly.

1.2. Caloric Restriction

The energy balance equation- the balance between the energy intake (energy in) and the energy expenditure (energy out) – has been known for several years as the equation to weight management. Excessive calorie intake regarding the physical activity performed is related to overweight/obesity (21, 46) and diet-related NCDs (28). In contrast, a reduction in energy intake and/or an increase in energy expenditure (through physical activity) promotes weight loss and it has shown effect in improving insulin sensitivity, fasting glucose levels and other cardiometabolic risk factors (47, 48).

Caloric restriction (CR) has been described as a reduction in energy intake, compared with the amount of calories that would be consumed *ad libitum* (AL), without causing malnutrition - guaranteeing the appropriate intake of micro and macronutrients ("undernutrition without malnutrition") (49-52). The restriction in the caloric intake varies widely, usually defined by a reduction of at least 10% in humans, varying from 20% up to 60% in animals (49, 53), of AL consumption

Moreover, different terms have been used to describe the same nutritional approach: caloric restriction and dietary restriction, are the most commonly used. During this work we will use caloric restriction, rather than dietary restriction, as the term indicates the nature of the approach. While dietary restriction also encompasses a broader scope of interventions, such as normo-caloric diets with specific micro or macronutrient restrictions, feeding pattern restriction (fasting) or restrictions related with food allergies or intolerances (51, 54, 55).

Caloric restriction has been studied since 1935, when McCay found that it increased lifespan in mice (56). Although has been widely accepted the effect of caloric restriction on increasing the lifespan, some authors criticise the fundament of caloric restriction studies and assumptions of extension of lifespan (57). Firstly, the use of the "extension of life span" expression suggests that there is an increase in life span over the biological longevity or even over the demographic trend. However, this is not what has been observed. Additionally, increase in longevity is not universal (58). The control animals, following (generally) an AL diet, are more prone to energy imbalance and to be overweight, and weight gain is associated with an early onset of age-related diseases and mortality. Thereby, as the author argues, caloric restriction does not increase longevity *per se*: rather the AL feeding shorts the lifespan" (59).

However, behind being acclaimed for many as the only known method to increase longevity in short lived species. a caloric restricted diet, mostly in animals, has been associated with numerous other health benefits, from reduced incidence of different types of cancer, improved immune system or even to reversion of MetS and insulin resistance (54). The most studied model organisms in caloric restriction are yeast, worms, fruit flies, mice and rats (60). Although the low translatability of studies in short lived species, they have been frequently used as the time required to longevity studies are low and consequently the costs are lower (60).

Data based on studies performed in rodents, in general found health benefits but are frequently based on short term interventions (61, 62). A four-month caloric restriction diet, in rats, reduced body weight and body fat, with a concomitant reduction in adipocyte size and stimulated lipid mobilization, compared to a control group following an AL diet. caloric restriction diet also up-regulated the adiponectin expression - an anti-inflammatory adipocytokine associated with improved insulin sensitivity - by adipocytes and an increased plasma adiponectin concentration. Inversely, the authors verified a significant decrease in insulin levels (61). A caloric restriction extended for a period longer than 6 months reduced the incidence of kidney disease in rats, compared with the AL group, additionally the beneficial effects were more evident when the extent of the restriction was higher than 40% of the AL intake, compared with a lower degree of restriction (63). An old-onset (18 months of age) caloric restriction in rats has beneficial effects, preventing the natural decrease of neurons, that are usually reduced with aging, even when applied in old age during 6 months, with no serious morphological consequences (62). The rodents seem to present a biphasic physical activity response when under a calorie restricted diet. Although it was expected a reduction on the physical activity to reduce the energy expenditure, it appears that initially there is an increase on the activity levels, followed by a chronic decrease in activity (54). The extension of lifespan in small rodents seems to have a linear relationship with the extent of the energy restriction up to 60%, a restriction higher than this seem to have a negative effect on lifespan (42).

Nonetheless, many studies in rhesus monkeys are being conducted, and apparently the effects are consistent with the effects found in rodents. In non-human primates, the effect of caloric restriction on lifespan requires further studies and time for the benefits to be established with any degree of certainty, mainly due to the high life expectancy with a

medium life span from 25 to 40 years (54). A caloric restricted diet in non-human primates improved glucoregulatory function and decreased risk factors for CVD and diabetes (64), such as lower weight and lower blood glucose levels (65), lower body fat and improved insulin sensitivity (60, 64, 65), increased high density lipoprotein-cholesterol (HDL-C) and lower triglycerides levels (60), when compared to the control group.

The onset age of a caloric restricted diet seems to be a crucial factor in the induced health effects in *rhesus monkeys* (66), however studies in rodents have been shown to have beneficial health effects even when started in adulthood (62), although the effects on life span are reduced compared to when the restriction is started earlier (54). Interestingly, the maturing primates keep gaining weight into adulthood and no negative implications in the immune system were noticed (65). Furthermore, caloric restriction did not compromise the reproductive system (64, 65).

There is an inherent loss of muscles and brain atrophy with ageing, a caloric restricted diet also enhanced these factors. Promoting a slower rate of muscle loss with age and preserving brain morphology, in non-human primates (54, 60, 64). Considering age related diseases, such as neoplasia and endometrioses, there was also a lower incidence in the caloric restricted group. Moreover, there was no apparent adverse effect on bone health (64). Effects of a caloric restricted diet on brain are not consensual. Divergent to the presented studies in *rhesus monkeys*, in grey mouse lemurs, caloric restriction affected the brain, accelerating the grey matter atrophy (volume reduction), yet, white matter was preserved in caloric restricted group compared to the control group. Even though the brain integrity was affected, no changes on the cognitive performances were perceived (67). This study, allowed to observe in non-human primates a positive extend on lifespan, when it is started in young adults as it was repeatedly observed in smaller animals. With a concomitant reduction in the risk of age associated diseases and mortality (67).

Regardless of the promising effect in animals, ethical and methodological barriers to apply such a long-term calorie-restricted study, as well as the low probability of adherence and long-term maintenance of diets make studies in humans scarce. In some parts of the world, naturally occurring caloric restriction in humans is not unusual. However, in these populations is frequently associated to malnutrition - poor diets, lacking micronutrients and proteins and commonly associated with impaired

development and abnormal organism function (68). Thus, few studies on high quality calorie restricted diets in humans are available. One of the first reports relating low-calorie intake with lifespan in humans was in the Okinawan population (69). Okinawans consume a nutrient-dense diet low in calories, having the lowest calorie intake in Japan. Characterized by reduced morbidity and mortality, and the greatest percentage of centenarians in Japan, had been described as a healthy longevity. The death rates due to heart disease, stroke, and cancer were approximately 30–40% lower compared to the rest of Japan and even higher when compared to the United States (69). Studies involving groups following certain lifestyles based on their own beliefs are another source of information to obtain nutritional data in humans. The Caloric Restriction Society (CRS)[1] consists of a group of individuals who practice caloric restriction with optimum nutrition (CRON) intending to improve their health and living longer. Comparing 18 CRS members, who had been practicing caloric restriction for an average of 6 years, and 18 healthy age-matched individuals eating typical American diets, similarly with previous results in studies conducted in animals, the caloric restricted group had a lower BMI and a lower total body fat. Total serum cholesterol, low density lipoprotein-cholesterol (LDL-C) were markedly lower, while HDL-C was higher in the caloric restricted than in the comparison group. Fasting plasma insulin and glucose values were also significantly lower, as well as BP (70). However, in both situations, is not possible to exclude that health benefits result from other factors that characterize those groups.

In non-obese adult humans, after a caloric restriction intervention, were observed reductions in body weight and BMI fasting glucose and insulin values, lower levels of total cholesterol, LDL-C, triglycerides, C-reactive protein (CRP) values, BP and an increase in HDL-C. Suggesting that, in humans, caloric restriction may be protecting against atherosclerosis and CVD risk (52).Additionally, caloric restriction seems to be more effective concerning BMI and weight reduction, compared to physical active and sedentary control groups, although insulin sensitivity was higher among both caloric restricted and physical active groups (52). In healthy overweight, glucose-tolerant adult subjects, caloric restricted diet and a similar caloric deficit – though diet combined with exercise – improve similarly the insulin sensitivity(71).

[1] Caloric Restriction Society website: https://www.crsociety.org

Moreover, the Nicoll and Henein (72) review, beyond highlighting the beneficial effects of caloric restriction in lowering BP even in short studies, also demonstrate that those who are normotensive following a caloric restricted diet are unlikely to suffer hypotension, usually showing no effect regarding BP.

The CALERIE (Comprehensive Assessment of Long-term Effects of Reducing Intake of Energy) trials[2] were the first controlled clinical trials of caloric restriction with an adequate nutrient provision in healthy, non-obese humans. Was conducted in two phases, pilot feasibility and safety trial in 2005, followed by phase 2, a three-site randomized controlled trial, with 2 years duration (73).In general, during the first phase of the trial, body weight and fat were reduced, carbohydrate metabolism and insulin sensitivity were improved. Also, a decline in markers of oxidative stress, serum concentrations of leptin, LDL-C, and CRP and increased adiponectin levels were observed (50).

The second phase, consistently with studies in animals, demonstrates that mild caloric restriction - the achieved restriction was 12%, less than half of the outlined (25%) -, improves cardiometabolic risk factors, even in young and middle-aged adults, who had normal risk factor at baseline (50, 74, 75).

A concern with caloric restriction in humans is a severe restriction with consequent excessive fat and lean mass loss, leading to health complications, such as hypotension and bradycardia, infertility and amenorrhea, osteoporosis, loss of strength, sarcopenia and decreased cardiac muscle mass, slower wound healing and impaired cell-mediated immunity, weakness, dizziness, lethargy, irritability, depression, and emotional deadening (52, 57). This can be even more relevant depending on the starting weight and the tissues that are catabolized during an energy restriction (52, 58). As for obese the beneficial effects are evident, in non-obese mice, weight loss can be dangerous and is inversely related with lifespan (58). Suggesting that the effects may not be mediated by lowered energy intake *per se* but by weight loss and achievement of a healthier body weight (59).

Despite the mechanism by which a caloric restriction brings such benefits is not fully understood (55), pharmacological studies have been done to try to find a compound that

[2] The CALERIE website: https://calerie.duke.edu

can mimic some of these effects (54, 57, 76), as the implementation of a caloric restricted diet for long periods is expected to be unsustainable.

The complexity of nutritional recommendations is stressed by the relationship of caloric intake and all causes mortality, similarly to BMI which also has a U-shaped association with mortality (77). In a Public Health point of view nutrition risk is always U-shaped, increasing difficulties in population-based approaches. As it becomes even more clear in countries with double burden of malnutrition, undernutrition, or overweight and/ or obesity. It is not possible to stablish an absolute safe standardized value for calorie intake.

1.3. Dietary Patterns - EPITeen

Dietary patterns have been used increasingly recently to evaluate diet-disease association in nutritional epidemiology, complementing the traditional single nutrient or food approach, as it has some recognized limitations. It fails to take in cumulative effects or inter-correlations between nutrients or foods. While dietary patterns analysis permit to study the overall diet, accounting for interactions and synergetic effects (78-81). The dietary pattern approach is even more relevant when different dietary exposures are simultaneously associated with a disease risk, potentially with opposite effects. However, if a single dietary exposure affects disease, it effect may be diluted in dietary pattern analysis (80, 81).

Furthermore, dietary patterns are easier for the general population to interpret or/and translate into diets, as in the reality, people do not eat isolated nutrients or food, but a complex diet (78).

Dietary patterns can be derived *a posteriori* using data driven approach – principal component analysis, factor or cluster analysis -, and *a priori* using hypothesis-driven approach – indexes or scores-, or a combination of the two – reduced rank regression or partial least squares (78-81). Cluster analysis groups individuals into mutually exclusive clusters, and it is easier to interpret than factor analysis, as individuals are assigned for a specific pattern, rather than having a score for each factor. However, both methods for pattern analysis have an inherent subjectivity, that might impact the number and type of pattern derived (81, 82).

The EPITeen cohort (Epidemiological Health Investigation of Teenagers in Porto) is a population based study that followed since 2003, a sample of adolescents that were born in 1990 (83). The participants were regularly evaluated during adolescence and at adult life. As part of the EPITeen cohort, and based on the Food Frequency Questionnaire performed at baseline (13y) were identified four dietary patterns by cluster analysis: the *Healthier*, the *Dairy Products*, the *Fast Food & Sweets* and the *Lower Intake* dietary pattern (84).

The *Lower Intake* dietary pattern, was the most prevalent dietary pattern at 13 years old (n=596; 40%) and was characterized by a lower consumption of the majority of the food groups and by significantly lower energy intake [1811.9 (378.3) kcal/d], compared with the other patterns (84). The *Healthier* dietary pattern (n=239; 16.1%) and the *Dairy*

Products dietary pattern (n=442; 29.7%) had an energy intake of 2724.4 (487.5) kcal/d and 2621.3 (362.3) kcal/d, respectively, while the "Fast food and Sweets" presented the highest energy intake [3343.2 (482.1) kcal/d] (84).

Additionally, the dietary patterns identified at 13 years old seems to track into young adulthood, although the differences between the identified groups were attenuated from 13 to 21 years old. As those belonging to the "Lower Intake" dietary pattern at 13 years old still have the lowest energy intake at 21 years old (85), it suggests that this group of participants have a consistent lower energy intake over the eight years of follow up.

2. Objective

Objective: With this work we intend to understand how a dietary pattern characterized by a low caloric intake during adolescence affects the cardiometabolic risk profile in young adulthood.

3. Methods

3.1. Participants

Data was collected as part of the EPITeen study, an Epidemiological Health Investigation of Teenagers in Porto, a population-based cohort (83). The participants, 13 years old adolescents born in 1990, were enrolled in public or private schools of Porto. They were evaluated at 13, 17, 21, 24 and at 27 years old, and the evaluation comprised a standardized questionnaire and a physical examination.

The study complies with the Declaration of Helsinki and the Ethic Committee of Hospital S. João and the Ethic Committee of the Institute of Public Health from the University of Porto approved the research protocol. Written informed consent was obtained from parents or legal tutors and adolescents in the waves performed under 18 years old (13 and 17 years old), and from participants at 21 years and later.

This longitudinal study will include data from first (13y) and third evaluations (21y), once that these are the waves with more extensive dietary information evaluation.

At baseline there were 2786 eligible adolescents, 2159 agreed to participate (77,5%). From those, 1489 (65.5%) had dietary information that allowed to identify dietary patterns at 13 years old (84). Considering those with attributed dietary pattern (n=1489), were excluded participants without complete blood analysis (n=527; 35.4%). Thus, the cross-sectional analysis at 13 years old was based on 962 (64.6%) participants. Considering those 962 participants, 677 (70.4%) were revaluated at 21 years old evaluation. As 59 (8.7%) were outliers (if calories more than 3 times de interquartile range, if fruit equal or higher 1.5 the interquartile range or if vegetables equal or higher 1.5 the interquartile range) and 11 (1.6%) did not have complete data on blood analysis, this analysis included 607 (89.7%) participants at 21y, see Figure 3.1..

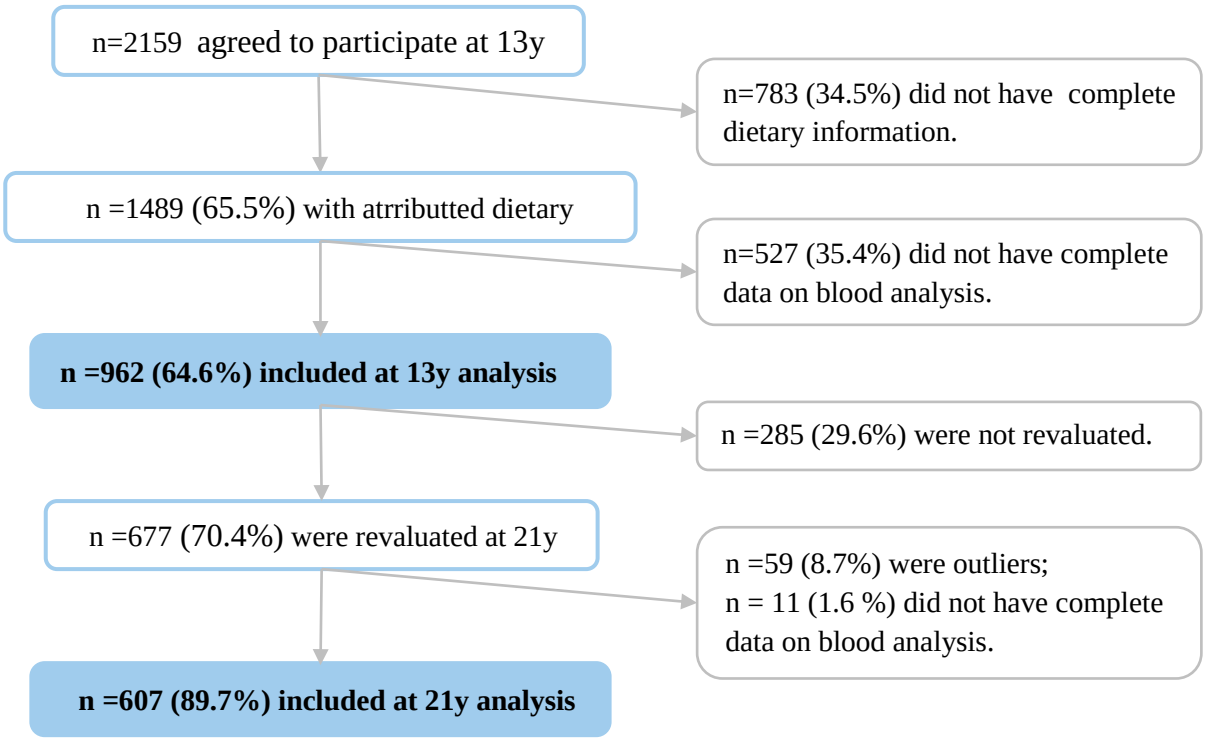

Figure 3.1. Participants included and excluded from the analysis at 13- and 21-years old waves, considering the EPITeen participants at 13 years old (n=2159).

3.2. Dietary, Lifestyle and Anthropometric variables

3.2.1. Dietary

Dietary information was assessed through a semi-quantitative food frequency questionnaire (FFQ) (86) regarding the previous 12 months, adapted and validated for the Portuguese adult population (87) and adapted for adolescents to use on the evaluation at 13 years old (88). Foods more frequently eaten by this age group were included on the FFQ, it comprised 91 food or beverage items and a frequency section with nine possible responses ranging from never to 6 or more times a day. Additionally, it also included an open-ended section for foods not listed in the questionnaire but eaten at least once a week (88).

At 13 years the FFQ was completed at home by the adolescent with the help of the parents or legal tutors (84). At 21 years old, the FFQ previously validated to the

Portuguese adult population (87), was applied by interviewer trough a face-to-face interview at the University department. The questionnaire included 86 food items and also an open-ended section for foods not listed in the questionnaire but eaten at least once a week (87).

At both ages, food and beverages were combined into 14 food groups (dairy products, seafood, red meat, white meat, pasta/potatoes/rice, cereals, soup, vegetables/legumes, fruit, added fats, fast foods, sweets and pastry, soft drinks and coffee/tea), according to nutritional similarities, as previously described (88). Based on the intake of the food groups, four dietary patterns: "Healthier" (n=239; 16.1%), "Dairy products" (n=442; 29.7%), "Fast food & Sweets" (n=212; 14.2%), and "Lower Intake" (n=596; 40%) were identified at 13 years old, by cluster analysis as described elsewhere (84).

3.2.1.1. Evaluation of Misreport

Energy intake (EI) misreport was evaluated using the Goldberg method (89) later corrected by Black (90). For each participant , at both ages, the ratio between the reported energy intake (repEI) and estimated basal metabolic rate (estBMR) (repEI:estBMR) was compared against the 95% confidence interval for physical activity level (PAL) (cut-offs), to identify potential misreporters, at both ages.

The repEI is likely to represent valid data – participants defined as plausible reporters - when the repEI:estBMR ratio is between de lower and upper cut-off:

Lower cut-off: $\text{PAL}*\exp\left[SDmin * \frac{S/100}{\sqrt{n}}\right]$

Upper cut-off: $\text{PAL}*\exp\left[SDmax * \frac{S/100}{\sqrt{n}}\right]$

Participants were identified as under reporters if repEI:estBMR ratio was below the lower cut-off and identified as over reporters if repEI:estBMR ratio was above the upper cut-off.

The repEI was based on the FFQ regarding the past 12 months, at both ages. The estBMR (kcal/day) was calculated according age and sex specific Schofield equations (91), using weight in kg and height in meters (Table 4.1.).

Table 4.1. Schofield equations for estimation of BMR (kcal/d) (91).

Gender/Age	EstBMR (Kcal/d)
Males	
10-17 years	16.2 * Wt + 137 * Ht + 516
18-29 years	15.0 x Wt – 10 * Ht+ 706
Females	
10-17 years	8.4 * Wt + 466 * Ht + 200
18-29 years	13.6 * Wt +283 * Ht+ 98
Abbreviations: Wt, Weight; Ht, Height	

PAL was assessed individually, based on a single question about leisure times. The question applied at 13 years has been previously validated by accelerometery to evaluate physical activity in this age group (92). A question including four response options was applied and later combined in three categories, according sex (the less active categories among girls and the more active categories among boys were aggregated, as described (92). Similarly, at 21 years old, a single question regarding leisure times was applied.

Thus, at both ages, participants were ranked according leisure time physical activity and then were assigned to PAL according to European Food Safety Authority (EFSA) Panel on Nutrition, Novel Foods and Food Allergens (NDA) (93): 1.6, 1.8 and 2.0 to low, moderate and vigorous physical activity, respectively, at 13 years old and 1.4, 1.6 and, 1.8 to low, moderate and vigorous physical activity, respectively, at 21 years old.

SDmin was -2 and SDmax was 2 for the 95% confidence lower and upper limits, respectively. As repEI was evaluated at individual level, n=1was considered to calculate the cut-offs.

S is given by the equation:

$$S = \sqrt{\frac{CV_{wEI}^2}{d} + CV_{wB}^2 + CV_{tP}^2}$$

Where CV_{wEI} is the within subject variation in energy intake, *d* is the number of days of diet assessment, CV_{wB} is the within-subject variation in repeated BMR measurements or

the precision of estimated estBMR compared with measured BMR and CV_{tP} is the between-subject variation in PAL.

As the repEI was obtained through FFQ that covers intra individual variability, CV_{wEI}=0 and *d*=365 were assumed. For CV_{wB} and CV_{tP} the revised factors by Black (90) were applied, CV_{wB}= 8.5% and CV_{tP}=15%.

3.2.2. Anthropometrics

In all the evaluations, the anthropometric measures were obtained with the participant standing in light indoor clothes and no shoes, by a trained observer according to international guidelines (94). Weight was measured in kilograms, to the nearest tenth, an body fat (%) was estimated by foot-to-foot bioelectrical impedance using a digital scale (Tanita TBF-300, Tanita Corporation of America, Inc, Illinois, USA) and height was measured in centimetres, to the nearest tenth, with the head of the participant in the Frankfurt plane. Waist was measured to nearest 0.1 cm with a flexible and non-distensible tape, avoiding exertion of pressure on the tissues, measured midway between the lower limit of the rib cage and the iliac crest, at the end of gentle expiration.

BMI was calculated as weigh (kg) divided by the square height (m). At 13 years old, adolescents were classified according to the age- and sex-specific BMI reference z-scores developed by the World Health Organization (WHO) (95) in four categories: thinness ($z < -2SD$), normal ($-2SD \leq z \leq +1SD$), overweight ($+1SD < z \leq +2SD$) and obesity ($z > +2SD$). At age of 21, WHO classification for adults was used (96): underweight, <18.5 kg/m^2; normal-weight, ≥18.5 and <25 kg/m^2; overweight, ≥25 and <30 kg/m^2; obese, ≥30 kg/m^2.

3.2.3. Cardiometabolic risk factors

At 13 years old, blood pressure was measured with a mercury sphygmomanometer using the auscultatory method, following the recommendations of the American Academy of Pediatrics (40). Two blood pressure measurements were taken, after resting for 10 minutes, separated by at least 5 min. A third measure was taken when the difference

between the first two was higher than 5mmHg. The average of the two closest measurements was used in this analysis.

At 21 years old, blood pressure was measured using an oscillometric method (OMRON Blood Pressure Monitor, M6 Comfort), according to standardized procedures. After 10 minutes of rest, two blood pressure measurements were taken, separately by at least five minutes; a third measure was taken if the difference between the first two was higher than 5 mmHg. The analysis used the average of the two closest measurements.

At both ages, an overnight fast intravenous blood sample was taken from an antecubital vein. Glucose was measured using conventional methods with an Olympus AU5400R automated clinical chemistry analyser (Beckman-CoulterR). Insulin was measured by electro chemiluminescent immunoassay using a CobasR e411 automated analyser (RocheR). All determinations took place in the Clinical Pathology Department of the Sao Joao Hospital Centre, Porto.

Also the Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) (97) was used, at both ages, as a marker of insulin resistance, based on fasting glucose and insulin concentrations:

$$\text{HOMA} - \text{IR} = \frac{\text{insulin } (\mu\text{U/ml}) * \text{glucose } (\text{mg/dl})}{405}$$

3.2.3.1. Metabolic syndrome definition:

At 13 years old, MetS was defined according to the National Cholesterol Education Program - Adult Treatment Panel III (NCEP ATP III) definition adapted for age (40) At least three of the following risk factors must be present: waist circumference ≥ 75th according to age and sex; triglycerides ≥ 100 mg/d; HDL- C < 50 mg/dL and/or Fasting Glucose ≥ 110 mg/dL and/or BP> 90th according to age, sex, and height.

At 21 years old MetS was defined according the NCEP ATP definition (32). At least three of the following risk factors should be present: waist circumference >102 cm for men and >88 cm for women; triglycerides ≥ 150 mg/dL; HDL-C <40 mg/dL for men and <50 mg/dL for women; Fasting Glucose ≥ 110 mg/dL; BP ≥ 130/85 mmHg.

3.2.4. Covariates

Participants leisure time activities were self-reported trough questionnaire, at both ages. At 13 years old was used a single question, validated in this age group, with four response options which were afterward combined in three categories- "most of times sitting", "most of times standing and/or walking", "most of the times active/very active" -, according to sex, as described (92). At 21 years old was used a single question with three answer options, corresponding to the same categories above described.

Time spent in sedentary activities (watching TV, playing computer and/or PlayStation, reading, and/or doing homework) at week (min/day) and weekend (min/weekend) was self-reported thought questionnaire and based on that, a mean time spent in sedentary activities per day was calculated (min/day). Participants were also asked if they had ever been diagnosed with diabetes by a doctor.

Education level of the parents was measured as the number of completed school years, and it was used as an indicator of socioeconomic status (SES), once that as previous studies demonstrated (43), it is highly correlated with the type of school that adolescents were attending (public or private), and with mother's occupation, classified as white or blue collar. The parent with higher education was considered to classify the participants.

Parents BMI was calculated based on self-reported weight and height and classified according WHO definition (96).

3.3. Statistical Analysis

Data are presented as counts (percentages) or mean (standard deviation). Proportions are compared with Chi square test or Fisher exact test, when appropriated. To compare the anthopometric measures and cardiometabolic risk factors, according to the dietary patterns, the analysis of variance (ANOVA) was used. To estimate adjusted means and respective 95% Confidence Interval (CI), according to the dietary patterns, the analysis of covariance (ANCOVA) was used. Satistical analysis was performed usig IBM SPSS statistics 24.0, considering a bilateral significance level of 0.05.

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4. Manuscript

ABSTRACT

Caloric restriction has been intensely studied as it seems to increase lifespan and delay diseases onset in rodents and short-lived species. Caloric restriction has also been associated with improved cardiometabolic risk factors, either in non-human and in human primates. However, available data are based on studies with too short period of follow-up and very restrictive or specific diets. We aimed to evaluate how the adherence to a dietary pattern characterized by a lower caloric intake at adolescence (13 years old) affects cardiometabolic risk factors on young adulthood (21 years old).

The study was based on the EPITeen cohort, considering participants with valid dietary information at 13 years old (n=962). At both waves diet was evaluated by a food frequency questionnaire, body mass index, blood pressure, insulin, triglycerides, and glucose were also assessed. The metabolic syndrome features were defined according to the National Cholesterol Education Program Adult Treatment Panel III definition in adults and an adaption was used at 13 years old. The Homeostatic Model Assessment for Insulin Resistance was used to assess insulin resistance. Four dietary patterns were previously identified at 13 years old: *Healthier* (n=239; 16.1%), *Dairy products* (n=442; 29.7%), *Fast food & Sweets* (n=212; 14.2%) and *Lower Intake* (n=596; 40.0%).

The mean daily energy intake considering all the participants was 2394.4 Kcal/d at 13 years old and 2279.6 Kcal/d at 21 years old, respectively. The *Lower Intake* dietary pattern has the lowest mean energy intake at both ages, 1806.5 Kcal/d and 2180.8 Kcal/d at 13 and 21 years old, respectively. In the cross-sectional analysis, adolescents belonging to the *Lower Intake* dietary pattern presented the lower values of glucose and insulin, triglycerides, and blood pressure. However, the differences were only statistically significant for glucose and systolic blood pressure after excluding the misreporters. No significant effect was found in the longitudinal approach.

Our data supports that a dietary pattern characterized by a lower energy intake may contribute to a better cardiometabolic profile, by promoting better glucose metabolism parameters and lower systolic blood pressure. These results become clearer after excluding the potential misreporters.

Keywords: adolescents; caloric restriction; cardiometabolic risk factors; cohort; energy intake; misreport; young adults

INTRODUCTION

Cardiovascular diseases (CVD) are the leading death cause worldwide [1]. Are the main contributor to mortality and morbidity among the non communicable diseases (NCD) - a major public health challenge worldwide, with great socioeconomic impact [2] - and among individuals with type 2 diabetes (T2D) [3]. Those with T2D have up to threefold higher risk of developing CVD [3].

International Diabetes Federation estimated that, in 2017, 451 million adults had diabetes worldwide, with 50% of the cases remaining undiagnosed [3]. This number is expected to increase to 693 million, by 2045 [4]. World Health Organization (WHO) estimated, in 2016, that more than 1.9 billion adults were overweight, and over 650 million were obese [5]. Diabetes and overweight are no longer adult pathologies. Globally, 41 million children under the age of 5, and more than 340 million children and adolescents aged 5-19 years old were overweight/obese in 2016 [5]. Used to be known by adult-onset diabetes, T2D is, nowadays, increasingly diagnosed in children and adolescents [6], and is the most prevalent type of diabetes, despite being preventable [7]. The prevalence of T2D rises as the number of overweight and obese [3, 8], being designated “The Twin Epidemic” [9].

The main risk factors for CVD are frequently observed in simultaneous in the same individual – this cluster have been defined as the metabolic syndrome (MetS). It is estimated that approximately a quarter of the world’s adult population has MetS [10]. In children and adolescents, the prevalence is around 3% in the whole population, and approximately 30% in the obese children [11].

Caloric Restriction (CR) has been studied since 1935, when McCay found that CR increases lifespan in mice [12], thenceforward has been studied and associated with many other health benefits, namely reversion of MetS and insulin resistance [13]. CR has been described as a reduction in energy intake, compared with the amount of energy that would be consumed *ad libitum* (AL), without causing malnutrition - guaranteeing the appropriate intake of micro and macronutrients [14-17].

Data based on studies performed in rodents, in general, found health benefits, however, are frequently based on short term interventions [18, 19]. Compared with a control group following an AL diet, a CR diet in rats reduced body weight and body fat and decreased insulin levels [18], reduced the incidence of chronic kidney disease, when extended for a

period longer than 6 months [20], and prevented the natural decrease of neurons even with an old-onset of the diet [19]. The extension of lifespan and beneficial effects, in small rodents seems to have a linear relationship with the extent of the energy restriction up to 60%, a restriction higher than this seem to have a negative effect on lifespan [20, 21].

Nonetheless, two prospective studies in non-human primates are being conducted – at the National Institute of Aging [22] and at the University of Wisconsin [23]. Apparently, the effects are consistent with the effects found in rodents. A CR diet in non-human primates improved glucoregulatory function and decreased risk factors for CVD and diabetes [24], such as lower weight and lower blood glucose levels [25], lower body fat and improved insulin sensitivity [24-26], increased high density lipoprotein-cholesterol (HDL-C) and lowered triglycerides (TG) levels [26], when compared to the control group. The maturing primates keep gaining weight into adulthood and no negative implications in the immune system were noticed [25]. Furthermore, CR did not seems to compromise the reproductive system [24, 25], and enhances some ageing related alterations, as it promotes a slower rate of muscle loss and seems to preserve the brain morphology [13, 24, 26]. Although the effects of a CR diet on brain are not consensual [27].

Regardless of the promising effect in animals, studies in humans are scarce. The magnitude of the caloric restriction is one of the main issues in transposing the results found in animals to humans, due to ethical and methodological barriers, it is difficult to apply restrictions in caloric intake as extensive and for such a long time as in animals. The restriction in the caloric intake varies widely, in animals the restriction varies from 20% up to 60%, while in humans the restriction reported is usually around 10% [14, 28], of AL consumption.

One of the first reports relating low energy intake with lifespan in humans was in the Okinawan population [29]. Okinawans consumed a nutrient-dense diet lower in energy, having the lowest energy intake in Japan (energy consumption, approximately, 16.4% lower compared to the average Japanese) [29]. The death rates due to heart disease, stroke, and cancer were approximately 30–40% lower compared to the rest of Japan [29]. Another study compared Caloric Restriction Society (CRS) members with healthy age-matched individuals eating typical American diets, found that the CRS members had a lower Body Mass Index (BMI), total body fat, total serum cholesterol, low density

lipoprotein-cholesterol (LDL-C), fasting plasma insulin, glucose, and blood pressure (BP), while presented higher HDL-C. [30]. Both observational studies - Okinawan population and CRS - were developed in very specific populations, so is not possible to exclude that health benefits result from other factors that characterize those groups.

The CALERIE (Comprehensive Assessment of Long-term Effects of Reducing Intake of Energy) trial was the first controlled clinical trial of CR with an adequate nutrient provision over a period of two years, in healthy, non-obese, young and middle-aged adults [31]. The CR group, presented reduced body weight, fat and BP, improved carbohydrate metabolism and insulin sensitivity, a decline in markers of oxidative stress, serum concentrations of leptin, total cholesterol, LDL-C, TG, C-reactive protein (CRP), tumour necrosis factor (TNF) and Homeostatic Model Assessment for Insulin Resistance (HOMA-IR), while adiponectin and HDL-C were increased [15, 32, 33].

The EPITeen cohort (Epidemiological Health Investigation of Teenagers in Porto) [34] identified at baseline (13 years) a dietary pattern characterized by a lower energy intake [35]. The dietary patterns identified at 13 years old seem to track into young adulthood. Those belonging to the *Lower Intake* dietary pattern at 13 years old still have the lowest energy intake at 21 years old [36]. Suggesting that this group of participants have a consistent lower energy intake over the eight years of follow up. Thus, using data from this population-based cohort, we aimed to understand the effect of a dietary pattern characterized by a lower caloric intake during adolescence into young adulthood, on cardiometabolic risk factors.

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METHODS

Participants Selection:

Data was collected as part of EPITeen a population based-cohort that recruited 13-years-old adolescents born in 1990, enrolled in public or private schools of Porto [34]. Beyond the baseline evaluation (13y), the participants were evaluated at 17, 21, 24 and 27 years old, the evaluation comprised a standardized questionnaire and a physical examination.

The study complies with the Declaration of Helsinki and the Ethic Committee of Hospital S. João and the Ethic Committee of the Institute of Public Health from the University of Porto approved the research protocol. Written informed consent was obtained from parents or legal tutors and adolescents in the waves performed under 18 years old (13 and 17 years old), and from participants at 21 years and later.

In this analysis were included the 1489 (65.5%) participants with dietary information that allowed to identify dietary patterns at 13 years old [35]. For the cross-sectional, at 13y, were excluded participants without blood sample (n=527; 35.4%), thus were included 962 participants. There were no differences between included and excluded participants (Table 1). Considering the 962 included at 13y, 677 (70.4%) were revaluated at 21 years old evaluation, and this analysis included 607 (89.7%) participants of that age group, as 59 (8.7%) were outliers (if calories more than 3 times de interquartile range, if fruit equal or higher 1.5 the interquartile range or if vegetables equal or higher 1.5 the interquartile range) and 11 (1.6%) did not have complete data on blood analysis.

Dietary information:

A semi-quantitative Food Frequency Questionnaire (FFQ) [37] regarding the previous 12 months, validated for the Portuguese adult population [38] and adapted for adolescents [39] was applied. Foods more frequently eaten by this age group were included on the FFQ, it comprised 91 food or beverage items and a frequency section with nine possible responses ranging from never to 6 times or more a day, and it also included an open-ended section for foods not listed in the questionnaire but eaten at least once a week [39].

At 13y, the FFQ was completed at home by the adolescent with the help of the parents or legal tutor. At 21y, the questionnaire was applied by interviewer through a face-to-face

interview at the University department, comprising 86 food items and also an open-ended section for foods not listed in the questionnaire but eaten at least once a week.

Food and beverages were combined into 14 food groups according to nutritional similarities, as previously described [39]. Based on the intake of the food groups, four dietary patterns: *Healthier* (n=239; 16.1%), *Dairy products* (n=442; 29.7%), *Fast food & Sweets* (n=212; 14.2%), and *Lower Intake* (n=596; 40.0%) were identified at 13y, by cluster analysis as described elsewhere [35].

Misreport of Energy intake:

Energy intake (EI) misreport was evaluated using the Goldberg method [40], later corrected by Black [41]. The ratio between the reported EI (repEI) and estimated basal metabolic rate (estBMR) (repEI:estBMR) was compared against the 95% confidence interval for physical activity level (PAL) (cut-off values), for each participant included in the analysis, at 13 and 21 years old.

Participants were classified as plausible reporters if the repEI:estBMR ratio was between de lower and upper cut-off values for PAL, under reporters if repEI:estBMR ratio was below the lower cut off value for PAL and, over reporters if repEI:estBMR ratio was above the upper cut-off value for PAL.

The repEI was based on the FFQ, at both ages. The estBMR (kcal/d) was calculated according age and sex specific Schofield equations [42].

PAL was estimated, at both waves, based on the leisure time activity level assessed thought a previously validated question [43]. The question allowed to assign participants to low, moderate, and vigorous PAL: 1.6, 1.8 and 2.0, respectively, at 13 years old. And 1.4, 1.6 and, 1.8, respectively, at 21 years old, according to European Food Safety Authority Panel on Nutrition, Novel Foods and Food Allergens (EFSA Panel on NDA) [44]. The 95% confidence interval for each PAL was considered.

<u>Anthropometrics:</u>

In all the evaluations, the anthropometric measures were obtained with the subject standing in light indoor clothes and no shoes, by a trained observer according to

international guidelines (49). Weight (kg) and body fat (%) were estimated by foot-to-foot bioelectrical impedance using a digital scale (Tanita TBF-300, Tanita Corporation of America, Inc, Illinois, USA) and height was measured in centimetres with the head of the participant in the Frankfurt plane. Waist circumference (WC) was measured with a flexible and non-distensible tape, measured midway between the lower limit of the rib cage and the iliac crest, at the end of gentle expiration.

At 13y, adolescents were classified according to the age- and sex-specific BMI reference *z* scores developed by the WHO [45] in four categories: thinness ($z < -2SD$), normal ($-2SD \leq z \leq +1SD$), overweight ($+1SD < z \leq +2SD$) and obesity ($z > +2SD$). At age of 21, WHO classification for adults was used [46]: underweight, <18.5 kg/m2; normal-weight, ≥18.5 and <25.0 kg/m2; overweight, ≥25.0 and <30.0 kg/m2; obese, ≥30.0 kg/m2.

Cardio metabolic risk factors:

At 13 years old, blood pressure was measured with a mercury sphygmomanometer using the auscultatory method, following the recommendations of the American Academy of Pediatrics [47]. At 21 years old, blood pressure was measured using an oscillometric method (OMRON Blood Pressure Monitor, M6 Comfort), according to standardized procedures.

At both ages, an overnight fast intravenous blood sample was taken from an antecubital vein. Serum glucose, cholesterol, triglycerides, and HDL-C were determined using automatic standard routine methods. Serum insulin was measured using a 125I-labelled insulin radioimmunoassay method. All determinations took place in the Clinical Pathology Department of the São João Hospital Centre, Porto. The Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) [48] was used as a marker of insulin resistance, based on fasting glucose and insulin concentrations.

Metabolic Syndrome definition:

At 13 years old, MetS was defined according to the National Cholesterol Education Program - Adult Treatment Panel III (NCEP ATP III) definition adapted for age [47]. At least three of the following risk factors must be present: WC ≥ 75^{th} according to age and

sex; triglycerides ≥ 100mg/d; HDL-C < 50 mg/dL and/or Fasting Glucose ≥ 110 mg/dL and/or BP > 90th according to age, sex, and height.

At 21 years MetS was defined according the NCEP ATP III [49]. At least three of the following risk factors should be present: WC > 102 cm for men and > 88 cm for women; triglycerides ≥ 150 mg/dL; HDL-C < 40 mg/dL for men and <50 mg/dL for women; Fasting Glucose ≥ 110 mg/dL; BP ≥ 130/85 mmHg.

Covariates:

Participants leisure time activities were self-reported trough questionnaire. Time spent in sedentary activities (watching TV, playing computer and/or PlayStation, reading, and/or doing homework) was self-reported thought questionnaire, considering week days (min/day) and weekend (min/weekend). Based on that, a mean time spent in sedentary activities *per* day was calculated (min/day).

Information on previous diagnosis of Diabetes was based on a self-reported question on if they had ever been diagnosed with diabetes by a doctor.

Education level of the parents was measured as the number of completed school years and the parent with higher education was considered to classify the participant. Participants education level was based on data from the 21 years old evaluation, the last school year successfully finished was considered. Parents BMI was calculated based on self-reported weight and height, collected through questionnaires answered by parents, and classified according WHO definition [46].

Statistical Analysis:

Data are presented as counts (percentages) or mean (standard deviation). Proportions are compared with Chi square or Fisher, when appropriated. To compare the anthopometric measures and cardiometabolic risk factors according to the dietary patterns the analysis of variance (ANOVA) was used. To estimate adjusted means and respective 95% Confidence Interval (CI), according to the dietary patterns, the analysis of covariance (ANCOVA) was used. Satistical analysis was performed usig IBM SPSS statistics 24.0, considering a bilateral significance level of 0.05.

RESULTS

At 13 years old, dietary patterns, differ between each other regarding the mean (SD) daily energy intake ($p<0.001$). The *Lower Intake* presents the lowest [1806.5 (380.1) kcal/d] and the *Fast Food & Sweets* the highest [3425.7 (474.0) kcal/d] (Table 2). A sensitivity analysis demonstrated a prevalence of misreport over 30% at 13 years old, 24.3% (n=234) of the participants were identified as potential under reporters and 6.9% (n=66) as potential over reporters (Table S1). Considering the plausible reporters, at this age, the dietary patterns still differ regarding the mean (SD) daily energy intake ($p<0.001$), the *Lower Intake* presents the lowest [2041.9 (272.1) kcal/d] and the *Fast Food & Sweets* the highest [3263.1 (482.4) kcal/d] (Table 3).

The characteristics of the 13 years old participants according to the dietary patterns are described in Table S2. They differ regarding the sex of the participants ($p=0.041$), all but *Dairy Products* pattern present a higher proportion of females with a higher proportion of females belonging to the *Fast Food & Sweets* dietary pattern. They also differ regarding the BMI ($p=0.009$) and the time spent in sedentary activities ($p=0.036$). The *Fast Food & sweets* have the lowest prevalence of overweight/obese participants and the highest proportion of participants spending more time in sedentary activities.

Participants belonging to the *Lower Intake* pattern and to the *Fast Food & Sweets* patterns present the highest and lowest anthropometric measures, respectively. The differences are statistically significant regarding WC, body fat percentage, BMI *z* scores and BMI. No significant differences between dietary patterns were found regarding glucose metabolism, serum lipid levels, and BP (Table 2). The prevalence of MetS is similar regarding the dietary patterns ($p=0.986$), as well as the prevalence of the different components of the MetS (Table 2).

Repeating the cross-sectional analysis considering only those identified as potential plausible reporters (n=606) (Table 3). Although not statistically significant, the *Lower intake* pattern presents the lowest values for WC and BMI, although presents the highest for fat mass percentage. Regarding other cardiometabolic parameters, those in the *Lower Intake* dietary pattern present the best profile, being the difference statistically significant for the glucose levels and the systolic BP.

When adjusting to sex and BMI of the participants and to the education level of the parents (Table 4), the dietary patterns were similar regarding the glucose metabolism

parameters, the serum lipid levels and BP but were significantly different regarding the anthropometric measures, the *Lower intake* presents higher WC, fat mass percentage, BMI *z* scores, and BMI.

Applying the same adjustments but considering only the participants identified as plausible reporters (Table 4), the *Lower Intake* pattern presents the best glucose metabolism parameters. However, only statistically significant regarding the glucose levels. Similarly, the *Lower Intake* presents the best BP measures, but only the systolic BP differs significantly of the other patterns.

At 21 years old analysis, were included 607 (63.1%) of those included at 13 years old analysis (characteristics of those include and excluded of the analysis in supplements, Table S3). The participants included at 21 years old analysis differ significantly from those not included regarding the nutritional status, the dietary patterns and in the prevalence of MetS. Those not included tend to be more overweight/obese, to belong to the *Fast Food & Sweets* dietary pattern, to have a higher prevalence of MetS and to have less educated parents.

The characteristics of the 21 years old participants according the dietary patterns are described in supplements (Table S4). They were similar regarding all but their education level ($p=0.003$) and their parents education level ($p<0.001$). The participants from the *Healthier* and *Dairy Products* patterns have higher educated parents and are themselves more educated. While the *Fast Food & Sweets* pattern have the highest proportion of less educated participants and one of the highest proportions of less educated mothers, as well.

In the longitudinal analysis (Table 5), at 21 years old, the dietary patterns differ regarding the mean (SD) daily energy intake ($p=0.024$). The *Lower Intake* pattern presents the lowest [2180.8 (593.0) kcal/d] and the *Fast Food & Sweets* the highest [2392.4 (767.4) kcal/d] mean daily energy intake. Those from the *Lower Intake* had the highest anthropometric measures and those from the *Fast Food & Sweets* had the lowest (Table 5). No significant differences were found between dietary patterns regarding the glucose metabolism parameters, the serum lipid levels and BP. At this age, the prevalence of MetS was almost reduced to 0% across patterns except for the *Fast Food & Sweets* pattern (n=1, 1.5%). However, concerning the MetS components, the patterns differ regarding the proportion of participants above the threshold values of HDL-C ($p=0.021$), being those in the *Dairy Products* pattern who have the highest proportion.

A sensitivity analysis, at 21 years old, demonstrated that 21.3% (n=129) of the participants were potential under reporters and 8.2% (n=50) potential over reporters (Table S5). Repeating the analysis for those identified as potential plausible reporters (n=427) (Table 6), the dietary patterns did not differ regarding the daily energy intake, the anthropometric measures, the glucose metabolism parameters, the serum lipid levels, and BP.

After adjusting to participants sex, BMI and education level, the patterns were similar regarding the glucose metabolism parameters and BP levels, but significantly different regarding the anthropometric measures, the total cholesterol, and the LDL-C (Table 7). The participants belonging to the *Lower Intake* dietary pattern present the higher WC, fat mass percentage and BMI. Those belonging to the *Dairy Products* pattern present the highest values of total cholesterol, and LDL-C.

Concerning the sensitivity analysis, the pattern with higher proportion of under reporters is the *Lower Intake* at both ages, 49.1% and 25.6%, at 13 years as 21 years, respectively. While the pattern with higher proportion of over reporters at both ages is the *Fast food & Sweets*, 35.9% and 15.2%, at 13 years and 21 years (Table S6). The characteristics of the participants differ significantly regarding sex and nutritional status at both ages, according reporting classification (Table S1; Table S5). At both ages, the overweight/obese males were more frequently identified as under reporters. Participants identified as plausible reporter have more educated parents, at both ages, and tend to be themselves more educated (at 21y).

DISCUSSION

In this analysis of a population based-sample, of 13 and 21-years old boys and girls, based on data from EPITeen cohort, our cross-sectional analysis suggested that a lower energy intake may contribute to a better cardiometabolic profile, even after adjustment for BMI, sex, and education level of the parents. However, in the longitudinal approach we did not find any better measures concerning the *Lower Intake* dietary pattern.

At 13-years-old, the *Lower Intake* dietary pattern have the lowest mean energy intake, around 25% kcal/day less compared to the mean energy intake of the total sample. When considering only the plausible reporters, this difference is reduced to approximately 19% kcal/d less. However, as the CALERIE trial demonstrated, even a mild CR (a restriction of approximately 12% compared do AL group) had beneficial effects with improved cardiometabolic risk factors compared to the AL group [30]. Also, in primates, a moderate restriction of 20-30%, improved cardiometabolic profile, compared to the AL group [24].

At 21 years old, even though the *Lower Intake* pattern still have the lowest mean energy intake, the difference was reduced to around 4% kcal/d less than the mean energy intake of the total sample. This difference is reduced to 2% when considering only the plausible reporters at 21 years old, which may explain the lack of differences in the cardiometabolic profile between patterns at this age.

With this study, we did not find any significant differences across dietary patterns, except for anthropometric measures, the patterns differ significantly concerning nutritional status, at 13 and 21 years old. At both ages, the dietary pattern with higher proportion of overweight/obese participants was the *Lower Intake* - the pattern with the lowest energy intake. While the pattern with the higher proportion of normal/underweight participants was the "*Fast Food & Sweets*", the pattern with the highest energy intake at both ages. These may result of two main factors: the participants with a lower energy intake may have reduced the energy intake to reverse an existent problem of overweight. Thus, this may minimize the potential effect of a lower energy intake, as participants with metabolic changes due to previous lifestyle behaviours will be classified in the *Lower Intake* dietary pattern. This effect was attenuated after adjustment to BMI, however, we cannot exclude that might exist a residual confounding. Or this may also result of a higher proportion of under reporters among the participants belonging to the *Lower Intake* pattern. To better understand in which extent our results could be affected by

misreporting, we identified the potential plausible, under or over reporters, at both ages. The pattern with higher proportion of participants identified as under reporters was the *Lower Intake* and there is a higher proportion of overweight/obese participants among those identified as under reporters. As has been largely described, those who are overweight/obese tend to under report and those underweight tend to over report [50-53]. To minimize this effect, we did a sensitivity analysis, including only the participants identified as potential plausible reporters.

Considering the plausible reporters (n=616), at 13 years old, the *Lower Intake* dietary pattern, presents improved anthropometric measures, glucose metabolism parameters and BP measures, and, in general, even better measures than the other dietary patterns. Inversely, the *Fast Food & Sweets* that presented, in general, better glucose metabolism parameters and BP, when considering the plausible reporters, these measures are, in general, worse than the other patterns. The results obtained in this group show clearer that the participants belonging to the *Lower Intake* dietary pattern present a better cardiometabolic profile, suggesting that a true lower energy intake might contribute to that.

At 21 years old, although the *Lower Intake* pattern did not stand for any better values than the other patterns, when considering the plausible reporters, beneficial trends were observed, as the values improved more expressively in the *Lower Intake* pattern than the other patterns.

The lack of differences between patterns in the longitudinal approach, may be due to loss of follow up and a potential bias of selection, although this is not expected as the proportion of participants belonging to this pattern remained similar (around 41% at 13 and 21 years old), but there is a loss of statistical power due to sample size. Nevertheless, the *Lower Intake* pattern did not present a better cardiometabolic profile, independently of the statistical significance. We cannot exclude that at this is age there are changes in the dietary patterns, although the dietary patterns were identified in adolescence seem to track into adulthood, the differences between dietary patterns were attenuated between 13 and 21 years old [54]. The difference on the energy intake of the *Lower Intake* pattern compared to the energy intake of the sample decreased from 24.6%, at 13 years old, to 4.3%, at 21 years old. Thus, it is expected to not be enough to improve the

cardiometabolic risk factors or anthropometric measures, significantly over the other patterns, at 21 years old.

Additionally, the *Lower Intake* pattern presents, consistently, lower BP measures highlighted when considering the plausible reporters. This is relevant as blood pressure in adolescence and young adulthood have been associated with hypertension in adulthood [55], one of the main predictors of cardiovascular disease in adults.

In accordance with the literature the prevalence of EI under report is higher than over report [53]. There were significant differences among dietary patterns concerning reporting at 13 years old. At 21 years of age the differences are attenuated, however, misreporting apparent to have a similar trend over the years, as the pattern with higher proportion of under reporting was the *Lower Intake*, while the pattern with higher proportion of over reporting was the *Fast food & Sweets*, at both ages. Males under reported more, at both ages, differently of what has been described [50-52]. Moreover, our results highlight that the education level of the parents may influence the extent of misreporting, as well as the education level of the young adults.

Additionally, in accordance with the literature at both ages, the under reporters tend to self-describe their leisure-time as active/very active, compared with the other participants [56]. Under/normal weight females over report more at both ages and tend to self-describe their leisure-time activities as most of the time sitting. Thus, and once that the Goldberg accounts for PAL, we cannot discriminate if the individuals are true under or over reporters, or if they are over or under reporting PAL, respectively. As in this study we used a subjective measure to estimate PAL, objective measures would be more accurate, although harder to apply in such a large population study. However, the question we applied at 13 years has been previously validated by accelerometery to evaluate physical activity level in this age group [43]. On the other side, the characteristics of over reporters have not been as studied as under reporters once that the proportion is usually lower, but we do not consider it negligible, as it corresponds to 6.7% of our sample at 13 years old and 8.2% of our sample at 21 years old, and systematic over reporting cannot be excluded [53].

There are some limitations associated with Goldberg method, as it assumes that an individual of a given age, sex and body weight requires a minimum energy intake in order to maintain a particular lifestyle, thus assumes that: energy intake is equal to energy

expenditure. As at 13 years old adolescents are in a growth phase, is expected that the energy intake is higher than the energy expenditure (positive energy balance) to maintain their lifestyle. Although the increment in energy intake is not that expressive [52], at 13 years, the proportion of over reporting might be overestimated. However, the proportion of under reporting was the most expressive at this age. Additionally, this method has low sensitivity, identifying only 50% of the under reporters, and it cannot make distinction between degrees of misreporting [57]. Nevertheless, we used appropriated age and sex specific cut-offs, and, at both ages, we identified different PAL according to self-report leisure time activities, instead of assuming a sedentary lifestyle, increasing the sensitivity of the cut-off values [57].

The term "lower energy intake" is more appropriate than "caloric restriction" as the differences in the energy intake are not as expressive as those associated with the term "caloric restriction" [14, 28].

A cross sectional analysis with the participants that had attributed dietary pattern, blood analysis, and were not outliers, independently if they were included in 13 years analysis, was performed (Table S7). Once the results were similar, despite the loss of participants, we choose to do a longitudinal analysis.

Limitations and strengths: This study has some strengths, as it is a population-based cohort, from adolescence to the young adulthood. Anthropometric measures were obtained by trained health professionals and a validated FFQ was applied.

Some of the limitations of this study are inherent to the methodology used. The dietary assessment methods recall on memory, and at 13 years old the FFQ was self-administered, that itself may increase the bias. A larger sample size could minimize issues due to lack of statistical power, namely the losses of follow up. Additionally, from 13 years to 21 years old evaluation there was loss of adolescents with worst profile (more obese and with higher prevalence of MetS) and from lower socioeconomic levels, leading to a potentially more homogeneous group at 21 years old group, which might contribute to the lack of differences.

CONCLUSION

Our study supports that a dietary pattern characterized by a lower energy intake may contribute to a better cardiometabolic profile in adolescents, by promoting a better glucose metabolism and a lower systolic blood pressure. These results became more clear after excluding the participants identified as potential misreporters. The longitudinal approach does not allow us to conclude whether a dietary pattern characterized by a lower energy intake, identified in adolescence, affects the cardiometabolic profile in young adulthood.

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Table 1. Comparison of demographic and anthropometric characteristics at baseline (13y), among included (n=962) and excluded (n=527) participants.

		Included n = 962 (64.6%)		**Excluded** n = 527 (35.4%)		
		n (%)				p-value
Sex	Female	516	(53.6)	286	(54.3)	0.815
	Male	446	(46.4)	241	(45.7)	
Dietary Patterns	Lower Intake	401	(41.7)	195	(37.0)	0.107
	Healthier	143	(14.8)	96	(18.2)	
	Dairy Products	290	(30.1)	152	(28.8)	
	Fast food & Sweets	128	(13.3)	84	(15.9)	
BMI *z* scores *****	Underweight	10	(1.0)	8	(1.7)	0.645
	Normal weight	665	(69.3)	329	(70.6)	
	Overweight	195	(20.3)	87	(18.7)	
	Obese	89	(9.3)	42	(9.0)	
Diabetes Diagnosed	No	904	(99.7)	498	(99.2)	0.210
	Yes	3	(0.3)	4	(0.8)	
Mean time spent in sedentary activities (min/d)	≤120	28	(3.3)	12	(2.7)	0.924
	121-240	291	(34.6)	156	(34.7)	
	241-360	308	(36.6)	168	(37.4)	
	>360	215	(25.5)	113	(25.2)	
Leisure time activities. Most of the time…	… sitting	418	(45.5)	227	(45.8)	0.889
	… standing and/or walking	185	(20.1)	104	(21.0)	
	… active/ very active	316	(34.4)	165	(33.3)	
Parents education (years)	≤6	220	(23.1)	104	(19.9)	0.461
	7-9	199	(20.9)	106	(20.3)	
	10-12	265	(27.8)	151	(28.9)	
	>12	270	(28.3)	162	(31.0)	

Abbreviations: BMI, Body Mass Index

* According to WHO criteria for *z* scores. [58]

Table 2 Cross-sectional analysis at 13 years old. Comparison of mean (SD) anthropometric and cardiometabolic characteristics and the metabolic syndrome prevalence, according to dietary patterns identified at baseline.

		Dietary Patterns				
		Lower Intake n = 401 (41.7%)	**Healthier** n =143 (14.8%)	**Dairy Products** n = 290 (28.8%)	**Fast food & Sweets** n =128 (13.3%)	
13 years old		Mean (SD)				p-value
Energy Intake (Kcal/day)		1806.5[a] (380.1)	2730.0[b] (494.8)	2586.7[c] (338.1)	3425.7[d] (474.0)	<0.001
Anthropometric measures						
Waist (cm)		73.32 [a] (9.11)	72.82 [a,b] (9.41)	72.10 [a,b] (8.07)	70.61[b] (7.07)	0.016
Fat mass (%)		22.37 [a] (9.63)	20.77 [a,b] (9.35)	20.29 [b] (9.22)	20.23 [a,b] (9.15)	0.012
BMI *z* scores [α]		0.59[a] (1.13)	0.52[a,b] (1.07)	0.45[a,b] (1.05)	0.29[b] (0.93)	0.036
BMI (Kg/m^2)		21.41[a] (3.76)	21.10[a,b] (3.77)	20.79[a,b] (3.35)	20.23[b] (2.81)	0.005
Glucose metabolism parameters						
Glucose (mg/dL)		85 (10)	84 (8)	86 (9)	86 (9)	0.191
Insulin (µUI/ml)		8.0 (5.9)	8.2 (5.2)	7.7 (6.6)	8.2 (5.5)	0.821
HOMA-IR		1.7 (1.3)	1.7 (1.1)	1.7 (1.5)	1.8 (1.2)	0.912
Serum lipid levels (mg/dL)						
Total cholesterol		166 (31)	165 (32)	170 (32)	164 (29)	0.229
Triglycerides		64 (26)	67 (35)	64 (27)	66 (27)	0.727
HDL-cholesterol		49 (11)	49 (12)	50 (11)	49 (10)	0.309
Blood Pressure (mmHg)						
Systolic		113 (11)	113 (11)	114 (11)	112 (11)	0.365
Diastolic		68 (8)	68 (8)	68 (9)	70 (7)	0.114
		n (%)				p-value
BP (AAP) [Ω]	Normal	263 (66.2)	96 (67.1)	191 (66.3)	80 (63.5)	0.466
	Prehypertension	62 (15.6)	26 (18.2)	40 (13.9)	27 (21.4)	
	Hypertension	72 (18.1)	21 (14.7)	57 (19.8)	19 (15.1)	
MetS [ǂ]	No	343 (86.6)	124 (86.7)	249 (86.8)	109 (87.9)	0.986
	Yes	53 (13.4)	19 (13.3)	38 (13.2)	15 (12.1)	
Components of MetS:						
Waist circumference ≥ 75th [β]	No	283 (70.6)	103 (72.0)	213 (74.0)	101 (80.8)	0.153
	Yes	118 (29.4)	40 (28.0)	75 (26.0)	24 (19.2)	
Triglycerides ≥ 100 mg/dL	No	360 (89.8)	122 (85.3)	261 (90.0)	114 (89.1)	0.469
	Yes	41 (10.2)	21 (14.7)	29 (10.0)	14 (10.9)	
HDL- cholesterol < 50 mg/dL	No	183 (45.5)	69 (48.3)	134 (46.2)	62 (48.4)	0.911
	Yes	219 (54.5)	74 (51.7)	156 (53.8)	66 (51.6)	
Fasting Glucose ≥ 110 mg/dL	No	399 (99.3)	143 (100.0)	288 (99.3)	127 (99.2)	0.785
	Yes	3 (10.7)	0 (0.0)	2 (0.7)	1 (0.8)	
BP> 90th [Ω]	No	263 (66.2)	96 (67.1)	191 (66.3)	80 (63.5)	0.928
	Yes	134 (33.8)	47 (32.9)	97 (33.7)	46 (36.5)	

Abbreviations: BMI, Body Mass Index; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; HDL, High Density Lipoprotein; BP, Blood Pressure; MetS, Metabolic Syndrome.

Different superscript letters indicate significant differences among dietary patterns at $p < 0.05$ in the Tukey comparison.

[α] According to WHO criteria for *z* scores [58];

*HOMA-IR = insulin (µU/ml) * glucose (mg/dL) /405 [48];

[ǂ] Defined according to the ATP III adapted definition of de Ferranti et al. [47];

[β] According to age and sex. [59];

[Ω] According to age, sex and height [60].

Table 3 Cross sectional analysis at 13 years old. Comparison of mean (SD) anthropometric and cardiometabolic characteristics, according to dietary patterns, considering only those identified as plausible reporters (n = 616).

	Dietary Patterns								
	Lower Intake n = 186 (30.2%)		**Healthier** n =107 (17.4%)		**Dairy Products** n = 248 (40.3%)		**Fast food & Sweets** n =75 (12.2%)		
	Mean (SD)								p-value
Energy Intake (Kcal/day)	2041.9[a]	(272.1)	2726.2[b]	(441.0)	2585.2[c]	(311.9)	3263.1[d]	(482.4)	**<0.001**
Anthropometric Measures									
Waist (cm)	70.06[a]	(6.99)	72.54[b]	(9.04)	71.39[a,b]	(7.53)	71.38[a,b]	(7.15)	0.054
Fat mass (%)	22.31[a]	(9.05)	21.09[a,b]	(9.14)	20.43[a,b]	(9.43)	18.62[b]	(9.20)	**0.024**
BMI *z* scores*	0.23	(1.01)	0.49	(1.06)	0.37	(1.01)	0.37	(0.99)	0.182
BMI (Kg/m^2)	20.30	(3.16)	21.03	(3.77)	20.54	(3.19)	20.42	(2.90)	0.310
Glucose metabolism parameters									
Glucose (mg/dL)	84[a,b]	(10)	84[a]	(8)	86[a,b]	(8)	87[b]	(9)	0.012
Insulin (µUI/ml)	7.68	(5.88)	7.84	(4.73)	7.65	(6.71)	7.88	(5.31)	0.987
HOMA-IR [α]	1.61	(1.28)	1.62	(0.99)	1.65	(1.50)	1.72	(1.24)	0.942
Serum lipid levels (mg/dL)									
Total cholesterol	171	(32)	166	(32)	169	(32)	164	(26)	0.290
Triglycerides	64	(24)	65	(33)	65	(28)	67	(28)	0.880
HDL-cholesterol	51	(11)	50	(12)	51	(11)	48	(9)	0.340
Blood Pressure (mmHg)									
Systolic	110[a]	(10)	112[a,b]	(12)	113[b]	(11)	114[b,c]	(10)	0.039
Diastolic	67	(8)	67	(8)	68	(9)	69	(8)	0.188

Abbreviations: BMI, Body Mass Index; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; HDL, High Density Lipoprotein.

Different superscript letters indicate significant differences among dietary patterns at $p < 0.05$ in the Tukey comparison.

*According to WHO criteria for *z* scores. [58]

[α] HOMA-IR = insulin (µU/ml) * glucose (mg/dL) /405 [48].

Table 4 Estimated means (95% CI) of participants characteristics at 13 years old, adjusted for BMI of participants and education level of the parents, considering all the participants included in the 13 years old analysis (n=962) and considering the participants identified as potential plausible reporters (n=616

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	Dietary Patterns				
	Lower Intake n = 401 (41.7%)	**Healthier** n = 143 (14.8%)	**Dairy Products** n = 290 (28.8%)	**Fast food & Sweets** n = 128 (13.3%)	
n=962 (all 13y participants)	Mean (95% CI)				p-value
Anthropometric Measures*					
Waist (cm)	73.35[a] (72.50; 74.20)	72.90[b] (71.46; 74.34)	72.17 (71.16; 73.18)	70.57[a,b] (69.04; 72.09)	0.013
Fat mass (%)	22.41[a,b] (21.48; 23.34)	21.16 (19.59; 22.73)	20.49[a] (19.39; 21.59)	20.14[b] (18.49; 21.78)	0.023
BMI *z* scores	0.59[a] (0.48; 0.69)	0.51 (0.33; 0.69)	0.45 (0.32; 0.57)	0.28[a] (0.10; 0.47)	0.036
BMI (Kg/m^2)	21.42 (21.07; 21.77)	21.15 (20.56; 21.74)	20.83 (20.42; 21.25)	20.20 (19.58; 20.81)	0.005
Glucose metabolism parameters					
Glucose (mg/dL)	84.4[a] (83.5; 85.3)	84.5 (82.9; 86.0)	86.0[a] (84.9; 87.1)	85.5 (83.9; 87.2)	0.144
Insulin (µUI/ml)	7.8 (7.2; 8.4)	8.6 (7.6; 9.5)	8.0 (7.3; 8.6)	8.0 (7.0; 9.0)	0.644
HOMA-IR[α]	1.7 (1.5; 1.8)	1.8 (1.6; 2.0)	1.7 (1.6; 1.9)	1.7 (1.5; 1.9)	0.674
Serum Lipid levels (mg/dL)					
Total cholesterol	166.3 (163.2; 169.4)	165.1 (159.8; 170.3)	169.7 (166.1; 173.4)	164.8 (159.3; 170.3)	0.320
Triglycerides	63.8 (61.1; 66.6)	67.1 (62.5; 71.8)	64.6 (61.3; 67.8)	65.2 (60.3; 70.1)	0.689
HDL-cholesterol	48.9 (47.8; 50.0)	49.4 (47.5; 51.3)	50.4 (49.1; 51.7)	48.8 (46.8; 50.8)	0.371
Blood Pressure (mmHg)					
Systolic	112.7 (111.6; 113.8)	112.8 (110.9; 114.6)	114.0 (112.7; 115.3)	112.4 (110.4; 114.3)	0.422
Diastolic	67.5 (66.7; 68.3)	68.2 (66.8; 69.5)	68.4 (67.4; 69.3)	69.5 (68.0;70.9)	0.121
n=616 (plausible reporters at 13y)	n = 186 (30.2%)	n =107 (17.4%)	n = 248 (40.3%)	n =75 (12.2%)	
Glucose metabolism parameters					
Glucose (mg/dL)	84.1[a,b] (82.9; 85.4)	84.1 (82.4; 85.8)[c]	86.3 (85.2; 87.4)[b,c]	86.7 (84.7,88.7)[a]	0.017
Insulin (µUI/ml)	7.7 (6.9; 8.6)	8.0 (6.9;9.1)	7.9 (7.1; 8.6)	7.7 (6.4; 9.0)	0.983
HOMA-IR[α]	1.6 (1.4; 1.8)	1.7 (1.4; 1.9)	1.7 (1.5; 1.9)	1.7 (1.4; 2.0)	0.914
Serum Lipid levels (mg/dL)					
Total cholesterol	171.3 (166.8; 175.8)	166.3 (160.3; 172.4)	169.5 (165.6; 173.5)	164.3 (157.1; 171.4)	0.317
Triglycerides	64.0 (60.0; 67.9)	64.9 (59.6; 70.2)	64.9 (61.4;68.3)	66.4 (60.1; 72.6)	0.939
HDL-cholesterol	51.1 (49.5; 52.6)	51.2 (49.1; 53.2)	51.0 (49.6; 52.3)	48.3 (45.8; 50.7)	0.236
Blood Pressure (mmHg)					
Systolic	110.6[a,b] (10.9.1; 112.1)	111.8 (109.8; 113.8)	113.0[b] (111.7;114.3)	114.4[a] (112.0; 116.8)	0.030
Diastolic	66.9[a] (65.7;68.0)	67.5 (66.0; 69.1)	68.1 (67.0; 69.1)	69.3[a] (67.4; 71.2)	0.159

Abbreviations: BMI, Body Mass Index; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; HDL, High Density Lipoprotein.

Different superscript letters indicate significant differences among dietary patterns at $p < 0.05$ in the Tukey comparison.

[α] HOMA-IR = insulin (µU/ml) * glucose (mg/dL) /405 [48];

* Adjusted for parents education level.

Table 5 Longitudinal analysis (n=607), comparison of mean (SD) anthropometric and cardiometabolic characteristics and the metabolic syndrome prevalence at 21 years old, according to dietary patterns identified at baseline.

		Dietary Patterns				
		Lower Intake n = 254 (41.8%)	**Healthier** n =92 (15.2%)	**Dairy Products** n = 195 (32.1%)	**Fast food & Sweets** n =66 (10.9%)	
21 years old		Mean (SD)				p-value
Energy Intake (Kcal/day)		2180.8 (593.0)	2391.6 (961.1)	2317.4 (685.8)	2392.4 (767.4)	0.024
Anthropometric Measures						
Waist (cm)		77.99[a] (10.14)	77.05[a, b] (9.45)	77.56[a] (8.91)	73.97[b] (7.74)	0.021
Fat mass (%)		20.79 (9.12)	19.68 (8.42)	19.25 (7.98)	19.07 (8.33)	0.218
BMI (kg/m^2)		23.29[a] (4.07)	22.84[a, b] (3.47)	22.81[a, b] (3.36)	21.82[b] (3.02)	0.036
Glucose metabolism parameters						
Glucose (mg/dL)		83 (7)	83 (7)	83 (6)	83 (6)	0.948
Insulin (µUI/ml)		9.2 (5.2)	8.2 (3.8)	8.3 (4.8)	9.0 (4.4)	0.199
HOMA-IR[α]		1.9 (1.2)	1.7 (0.8)	1.7 (1.0)	1.8 (0.9)	0.194
Serum lipid levels (mg/dL)						
Total cholesterol		177 (34)	170 (32)	180 (33)	171 (31)	0.073
Triglycerides		86 (38)	84 (36)	85 (39)	83 (40)	0.930
HDL-cholesterol		57 (13)	55 (14)	57 (12)	57 (11)	0.588
LDL-cholesterol		103 (26)	98 (26)	106 (28)	98 (24)	0.066
Blood Pressure (mmHg)						
Systolic		107 (11)	109 (12)	109 (11)	108 (13)	0.516
Diastolic		69 (7)	69 (7)	68 (7)	68 (8)	0.959
		n (%)				
MetS ǂ	No	254 (100.0)	92 (100.0)	195 (100.0)	65 (98.5)	0.109
	Yes	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.5)	
Criteria of MetS:						
Central Obesity [β]	No	236 (92.9)	89 (96.7)	188 (96.4)	64 (97.0)	0.304
	Yes	18 (7.1)	3 (3.3)	7 (3.6)	2 (3.0)	
Triglycerides ≥ 150 mg/dL	No	234 (92.1)	88 (95.7)	183 (93.8)	62 (93.9)	0.677
	Yes	20 (7.9)	4 (4.3)	12 (6.2)	4 (6.1)	
HDL-c [Ω]	No	226 (89.0)	74 (80.4)	181 (92.8)	58 (87.9)	0.021
	Yes	28 (11.0)	18 (19.6)	14 (7.2)	8 (12.1)	
Fasting Glucose ≥ 110 mg/dL	No	253 (99.6)	91 (98.9)	195 (100.0)	66 (100.0)	0.556
	Yes	1 (0.4)	1 (1.1)	0 (0.0)	0 (0.0)	
BP ≥ 130/85	No	244 (96.1)	89 (96.7)	187 (95.9)	62 (93.9)	0.829
	Yes	10 (3.9)	3 (3.3)	8 (4.1)	4 (6.1)	

Abbreviations: BMI. Body Mass Index; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; HDL, High density lipoprotein; LDL, Low Density Lipoprotein; MetS, Metabolic Syndrome; BP, Blood Pressure.

Different superscript letters indicate significant differences among dietary patterns at $p < 0.05$ in the Tukey comparison.

[α] HOMA-IR = insulin (µU/ml) * glucose (mg/dL) /405 [48];
ǂ According the definition of NCEP ATP III - National Cholesterol Education Program - Adult Treatment Panel III [49];
[β] According to sex: waist circumference >102 cm for men and >88 cm for women;
[Ω] According to sex: <40 mg/dL for men and <50 mg/dL for women.

Table 6 Longitudinal analysis, comparison of mean (SD) anthropometric and cardiometabolic characteristics of the participants, at 21 years old, according dietary patterns, considering only those with a plausible report (n =427).

	Dietary Patterns				
	Lower Intake n = 171 (40.0%)	**Healthier** n =64 (15.0%)	**Dairy Products** n = 147 (34.4%)	**Fast food & Sweets** n =45 (10.5%)	
	Mean (SD)				p-value
Energy Intake (Kcal/day)	2257.6 (468.7)	2334.4 (555.4)	2330.2 (563.9)	2337.4 (527.9)	0.549
Anthropometric Measures					
Waist (cm)	75.96 (7.84)	74.67 (8.55)	76.38 (8.26)	73.43 (7.16)	0.121
Fat mass (%)	19.95 (8.53)	19.05 (8.14)	18.84 (7.79)	18.75 (7.30)	0.610
BMI (kg/m^2)	22.36 (3.08)	22.14 (3.19)	22.44 (3.07)	21.77 (2.34)	0.593
Glucose metabolism parameters					
Glucose (mg/dL)	83 (8)	82 (7)	83 (6)	82 (6)	0.529
Insulin (µUI/ml)	8.36 (4.12)	7.93 (3.46)	8.39 (5.06)	8.67 (3.70)	0.838
HOMA-IR [α]	1.73 (0.95)	1.61 (0.73)	1.73 (1.08)	1.76 (0.80)	0.820
Serum lipid levels (mg/dL)					
Total cholesterol	178 (32)	168 (34)	178 (33)	171 (28)	0.132
Triglycerides	85 (38)	83 (33)	86 (40)	83 (41)	0.942
HDL-cholesterol	59 (13)	57 (14)	58 (12)	57 (11)	0.637
LDL-cholesterol	102 (24)	95 (27)	103 (28)	98 (23)	0.148
Blood Pressure (mmHg)					
Systolic	106 (11)	107 (11)	108 (11)	107 (13)	0.617
Diastolic	68 (7)	68 (7)	68 (7)	68 (8)	0.990

Abbreviations: BMI, Body Mass Index; HOMA-IR. Homeostatic Model Assessment for Insulin Resistance; HbA1c, Glycated Haemoglobin A1c; HDL, High Density Lipoprotein; LDL, Low Density Lipoprotein.

[α] HOMA-IR = insulin (µU/ml) * glucose (mg/dL) /405 [48].

Table 7 Estimated means (95% CI) of participants characteristics, at 21 years of age, adjusted for sex, BMI, and participants education level.

	Dietary Patterns				
	Lower Intake	**Healthier**	**Dairy Products**	**Fast food & Sweets**	
	Estimated Mean (95% CI)				p-value
Anthropometric Measures *					
Waist (cm)	79.00[a] (77.71; 80.25)	78.16 (76.19; 80.14)	78.50[b] (77.00; 80.00)	75.57[a,b] (73.42; 77.72)	0.041
Fat mass (%)	20.77[a] (19.77; 21.94)	20.39[b] (18.83; 21.94)	20.04[c] (18.87; 21.21)	18.07[a,b,c] (16.37; 19.77)	0.039
BMI (kg/m^2)	23.55[a] (23.02; 24.08)	23.25 (22.42; 24.07)	23.17[a, b] (22.55; 23.79)	22.15[a] (21.25; 23.05)	0.049
Glucose metabolism parameters					
Glucose (mg/dL)	83.5 (82.6; 84.5)	83.5 (82.0; 85.0)	83.2 (82.1; 84.3)	83.6 (82.0; 85.2)	0.955
Insulin (µUI/mL)	8.7 (8.1; 9.4)	8.2 (7.2; 9.2)	8.4 (7.6; 9.1)	9.2 (8.1; 10.3)	0.416
HOMA-IR[α]	1.8 (1.7; 2.0)	1.7 (1.5; 1.9)	1.7 (1.6; 1.9)	1.9 (1.7; 2.1)	0.500
Serum lipid levels (mg/dL)					
Total cholesterol	173.6[a,b,c] (169.0; 178.2)	167.0 (159.9; 174.1)	178.0[b] (172.7; 183.4)	168.8 (161.0; 176.5)	0.022
Triglycerides	80.6 (75.2; 86.0)	78.3 (69.99; 86.6)	80.6 (74.3; 86.9)	78.6 (69.4; 87.8)	0.937
HDL-cholesterol	55.8 (54.2; 57.44)	54.0 (51.6; 56.5)	55.9 (54.0; 57.7)	54.1 (51.5; 56.8)	0.371
LDL-cholesterol	101.7[a, b] (97.9; 105.5)	97.3[a] (91.4; 103.2)	106.0[b] (101.6; 110.5)	98.9[a,b] (92.4; 105.4)	0.036
Blood Pressure (mmHg)					
Systolic	107.6 (106.3; 108.9)	108.9 (106.9; 110.8)	108.5 (107.0; 110.0)	110.3 (108.1; 112.4)	0.146
Diastolic	67.9 (66.9; 68.9)	68.2 (66.6; 69.8)	67.7 (66.5; 68.9)	68.8 (67.1; 70.5)	0.727

Abbreviations: BMI, Body Mass Index; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; HbA1c, Glycated Haemoglobin A1c; HDL, High Density Lipoprotein; LDL, Low Density Lipoprotein.

Different superscript letters indicate significant differences among dietary patterns at $p < 0.05$ in the Tukey comparison.

[α] HOMA-IR = insulin (µU/ml) * glucose (mg/dL) /405 [48];
* Adjusted for sex and participants education level.

SUPPLEMENTS

Table S1 Characteristics of baseline participants (13y) according reporting classification.

		Under report N =234 (24.3%)	Plausible Report N = 616 (64.0%)	Over report N =66 (6.9%)	
		n (%)			p-value
Sex	Female	86 (36.8)	356 (57.8)	55 (83.3)	<0.001
	Male	148 (63.2)	260 (42.2)	11 (16.7)	
BMI zscores	Under/Normal Weight	117 (50.0)	472 (76.6)	59 (89.4)	<0.001
	Overweight/ Obese	117 (50.0)	144 (23.4)	7 (10.6)	
Parents education (years)	≤6	59 (25.3)	134 (21.9)	14 (21.2)	0.320
	7-9	46 (19.7)	123 (20.1)	21 (31.8)	
	10-12	65 (27.9)	167 (27.3)	16 (24.2)	
	>12	63 (27.0)	187 (30.6)	15 (22.7)	
Mean time spent in sedentary activities (min/d)	≤120	6 (3.1)	18 (3.2)	3 (5.0)	0.895
	121-240	68 (34.7)	195 (35.0)	18 (30.0)	
	241-360	70 (35.7)	200 (35.9)	26 (43.3)	
	>360	52 (26.5)	144 (25.9)	13 (21.7)	
Leisure time activities. Most of the time…	… sitting	70 (29.9)	298 (48.4)	48 (72.7)	<0.001
	… standing and/or walking	42 (17.9)	133 (21.6)	10 (15.2)	
	.. active/ Very active	122 (52.1)	185 (30.0)	8 (12.1)	

Abbreviations: BMI, Body Mass Inex.

Table S2 Distribution of dietary patterns identified at baseline according to adolescent and parents' characteristics, at 13 years old (n=962).

		Dietary Patterns								
		Lower Intake n = 401 (41.7%)		**Healthier** n =143 (14.8%)		**Dairy Products** n = 290 (28.8%)		**Fast food & Sweets** n =128 (13.3%)		
		n (%)								p-value
Sex	Female	228	(56.9)	72	(50.3)	139	(47.9)	77	(60.2)	0.041
	Male	173	(43.1)	71	(49.7)	151	(52.1)	51	(39.8)	
BMI *z* scores*	Under/Normal Weight	261	(65.4)	102	(71.3)	209	(72.3)	103	(80.5)	0.009
	Overweight/ Obese	138	(34.6)	41	(28.7)	80	(27.7)	25	(19.5)	
Mean time spent in sedentary activities (min/d)	≤120	12	(3.4)	4	(3.1)	7	(2.7)	5	(4.6)	0.036
	121-240	120	(34.3)	49	(38.6)	101	(39.3)	21	(19.4)	
	241-360	128	(36.6)	50	(39.4)	86	(33.5)	44	(40.7)	
	>360	90	(25.7)	24	(18.9)	63	(24.5)	38	(35.2)	
Leisure-time activities Most of the time…	… sitting	187	(48.6)	55	(41.7)	123	(43.8)	53	(43.8)	0.684
	… standing and/or walking	76	(19.7)	29	(22.0)	53	(18.9)	27	(22.3)	
	… active/ very active	122	(31.7)	48	(36.4)	105	(37.4)	41	(33.9)	
Parents education (years)	≤6	115	(29.0)	18	(12.8)	48	(16.6)	39	(31.0)	<0.001
	7-9	83	(20.9)	22	(15.6)	54	(18.6)	40	(31.7)	
	10-12	117	(29.5)	43	(30.5)	78	(26.9)	27	(21.4)	
	>12	82	(20.7)	58	(41.1)	110	(37.9)	20	(15.9)	

Abbreviations: BMI, Body Mass Index

*According to WHO criteria for *z* scores. [58]

Table S3 Comparison of those included in the longitudinal analysis (Included) and those who were only included at 13 years old cross-sectional analysis (Not included), considering the baseline characteristics.

		Included n = 607 (63.1%)	**Not included** n =355 (36.9%)	p-value
		n (%)		
Sex	Female	321 (52.9)	195 (54.9)	0.539
	Male	286 (47.1)	160 (45.1)	
Dietary Patterns	Lower Intake	254 (41.8)	147 (41.4)	0.023
	Healthier	92 (15.2)	51 (14.4)	
	Dairy Products	195 (32.1)	95 (26.8)	
	Fast food & Sweets	66 (10.9)	62 (17.5)	
BMI z scores*	Under/Normal Weight	440 (72.8)	235 (66.2)	0.029
	Overweight/ Obese	164 (27.2)	120 (33.8)	
Mean time spent in sedentary activities (min/d)	≤120	21 (3.9)	7 (2.3)	0.234
	121-240	194 (36.3)	97 (31.5)	
	241-360	188 (35.2)	120 (39.0)	
	>360	131 (24.5)	84 (27.3)	
Leisure time activities. Most of the time…	… sitting	270 (46.6)	148 (43.5)	0.553
	… standing and/or walking	11 (19.2)	74 (21.8)	
	… active/ very active	198 (34.2)	118 (34.7)	
Metabolic Syndrome	No	530 (88.9)	296 (83.6)	0.019
	Yes	66 (11.19)	58 (16.4)	
Diabetes diagnosed	No	570 (99.7)	334 (99.7)	0.804
	Yes	2 (0.3)	1 (0.3)	
Parents education (years)	≤6	113 (18.7)	107 (30.7)	<0.001
	7-9	109 (18.0)	90 (25.8)	
	10-12	180 (29.8)	85 (24.4)	
	>12	203 (33.6)	67 (19.2)	

Abbreviations: BMI., Body mass index

* According to WHO criteria for zscores. [58];

Table S4 Distribution of Dietary patterns identified at baseline according young adults' (21 years old) and parents' characteristics (n =607).

		Dietary Patterns								
		Lower Intake n = 254 (41.8%)		**Healthier** n =92 (15.2%)		**Dairy Products** n = 195 (32.1%)		**Fast food & Sweets** n =66 (10.9%)		
		n (%)								p-value
Sex	Female	138	(54.3)	46	(50.0)	95	(48.7)	42	(63.6)	0.176
	Male	116	(45.7)	46	(50.0)	100	(51.3)	24	(36.4)	
BMI	Under / Normal Weight	187	(73.9)	71	(79.8)	143	(74.5)	55	(84.6)	0.241
	Overweight / Obese	66	(26.1)	18	(20.2)	49	(25.5)	10	(15.4)	
Last school year finished	≤9	20	(7.9)	6	(6.5)	8	(4.1)	10	(15.2)	0.003
	10-12	82	(32.3)	17	(18.5)	44	(22.6)	17	(25.8)	
	>12	152	(59.8)	69	(75.0)	143	(73.3)	39	(59.1)	
Mean time spent in sedentary activities (min/d)	≤120	21	(8.3)	5	(5.4)	9	(4.6)	4	(6.1)	0.191
	121-240	76	(29.9)	25	(27.2)	47	(24.1)	28	(42.4)	
	241-360	73	(28.7)	31	(33.7)	70	(35.9)	17	(25.8)	
	>360	84	(33.1)	31	(33.7)	69	(35.4)	17	(25.8)	
Leisure-time activities. Most of the time…	… sitting	86	(33.9)	32	(34.8)	58	(29.7)	25	(37.9)	0.418
	… standing and/or walking	124	(48.8)	38	(41.3)	105	(53.8)	28	(42.4)	
	… very active	44	(17.3)	22	(23.9)	32	(16.4)	13	(19.7)	
Parents education (years)	≤6	64	(25.3)	9	(9.9)	25	(12.8)	15	(22.7)	<0.001
	7-9	47	(18.6)	12	(13.2)	29	(14.9)	21	(31.8)	
	10-12	80	(31.6)	31	(34.1)	50	(25.6)	19	(28.8)	
	>12	62	(24.5)	39	(42.9)	91	(46.7)	11	(16.7)	

Abbreviations: BMI, Body Mass Index

Table S5 Participants characteristics at 21 years old (n=607) according reporting classification.

		Under reporters N = 129 (21.3%)	**Plausible Reporters** N = 427 (70.3%)	**Over reporters** N = 50 (8.2%)	
		n (%)			p-value
Sex	Female	50 (38.8)	240 (56.2)	31 (62.0)	0.001
	Male	79 (61.2)	187 (43.8)	19 (38.0)	
BMI	Under/Normal Weight	62 (48.1)	351 (83.6)	43 (86.0)	<0.001
	Overweight/ Obese	67 (51.9)	69 (16.4)	7 (14.0)	
Last school year finished	≤9	10 (7.8)	24 (5.6)	9 (18.0)	0.001
	10-12	41 (31.8)	101 (23.7)	18 (36.0)	
	>12	78 (60.5)	302 (70.7)	23 (46.0)	
Parents education (years)	≤6	30 (23.3)	70 (16.4)	13 (26.0)	0.136
	7-9	21 (16.3)	76 (17.8)	12 (24.0)	
	10-12	40 (31.0)	125 (29.3)	15 (30.0)	
	>12	38 (29.5)	155 (36.4)	10 (20.0)	
Mean time spent in sedentary activities (min/d)	≤120	13 (10.1)	23 (5.4)	3 (6.0)	0.445
	121-240	40 (31.0)	121 (28.3)	14 (28.0)	
	241-360	33 (25.6)	143 (33.5)	15 (30.0)	
	>360	43 (33.3)	140 (32.8)	18 (36.0)	
Leisure time activities. Most of the time…	… sitting	30 (23.3)	145 (34.0)	26 (52.0)	0.003
	… standing and/or walking	70 (54.3)	203 (47.5)	21 (42.0)	
	… active/ very active	29 (22.5)	79 (18.5)	3 (6.0)	

Abbreviations: BMI, Body Mass Index

Table S6 Reporting classification among the Dietary patterns, at 13 (n = 962) and 21 (n =607) years old, according Goldberg method.

	Dietary Patterns				
	Lower Intake	**Healthier**	**Dairy Products**	**Fast food & Sweets**	p-value*
13 years					
Under reporters	197 (49.1)	13 (9.1)	24 (8.3)	0 (0.0)	<0.001
Plausible Reporters	186 (46.4)	107 (74.8)	248 (85.5)	75 (58.6)	
Over reporters	0 (0.0)	12 (8.4)	8 (2.8)	36 (35.09)	
Missing	18 (4.5)	11 (7.7)	10 (3.4)	7 (5.5)	
21 years					
Under reporters	65 (25.6)	20 (21.7)	33 (16.9)	11 (16.7)	0.123
Plausible Reporters	171 (67.3)	64 (69.6)	147 (75.4)	45 (68.2)	
Over reporters	18 (7.1)	7 (7.6)	15 (7.7)	10 (15.2)	
Missing	0 (0.0)	1 (1.1)	0 (0.0)	0 (0.0)	

* p-value obtained through Chi-square comparison without accounting to the category “Missing”.

Table S7 Transversal analysis (n=862), comparison of mean (SD) anthropometric and cardiometabolic characteristics and the metabolic syndrome prevalence at 21 years old, according to the dietary patterns identified at baseline.

		Dietary Patterns				
		Lower Intake n = 347 (40.3%)	**Healthier** n =135 (15.7%)	**Dairy Products** n = 280 (32.5%)	**Fast food & Sweets** n =100 (11.6%)	
21 years old		Mean (SD)				p-value
Energy Intake (Kcal/day)		2129.0[a] (588.8)	2327.5[b] (873.1	2318.4[b] (703.4)	2302.1[b] (752.8)	0.002
Anthropometric Measures						
Waist (cm)		78.25[a] (10.46)	76.85[a,b] (9.10)	77.26[a,b] (8.84)	75.30[b] (9.80)	0.049
Fat mass (%)		20.72[a] (9.29)	19.70[a,b] (8.29)	19.28[b] (8.37)	19.95[a,b] (8.40)	0.225
BMI (kg/m^2)		23.42[a] (4.21)	22.68[a,b] (3.46)	22.70[b] (3.36)	22.30[b,c] (3.64)	0.018
Glucose metabolism parameters						
Glucose (mg/dL)		84 (11)	82 (7)	83 (6)	83 (7)	0.539
Insulin (µUI/ml)		9.36[a] (5.45)	8.29[b] (4.35)	8.54[b] (4.57)	9.01[a,b] (4.37)	0.082
HOMA-IR[α]		1.95[a] (1.29)	1.70[b] (0.91)	1.76[b] (0.98)	1.86[a,b] (0.94)	0.064
Serum lipid levels (mg/dL)						
Total cholesterol		177[a] (33)	170[b] (33)	181[a] (35)	176[a,b] (33)	0.026
Triglycerides		86 (38)	82 (35)	87 (41)	84 (40	0.623
HDL-cholesterol		57 (13)	55 (13)	58 (12)	57 (12)	0.393
LDL-cholesterol		102[a,b] (26)	98[a] (27)	106[b] (29)	102[a,b] (26)	0.062
Blood Pressure (mmHg)						
Systolic		108 (11)	108 (12)	109 (11)	108 (13)	0.309
Diastolic		69 (8)	67 (7)	69 (7)	68 (8)	0.270
						n (%)
MetS ǂ	No	344 (99.1)	135 (100.0)	280 (100.0)	98 (98.0)	0.086
	Yes	3 (0.9)	0 (0.0)	0 (0.0)	2 (2.0)	
Criteria of MetS:						
Central Obesity [β]	No	317 (91.4)	130 (96.3)	267 (95.4)	94 (94.0)	0.106
	Yes	30 (8.6)	5 (3.7)	13 (4.6)	6 (6.0)	
Triglycerides ≥ 150 mg/dL	No	319 (91.9)	128 (94.8)	262 (93.6)	93 (93.0)	0.697
	Yes	28 (8.1)	7 (5.2)	18 (6.4)	7 (7.0)	
HDL-c [Ω]	No	310 (89.3)	110 (81.59	259 (92.5)	87 (87.0)	0.009
	Yes	37 (10.7)	25 (18.5)	21 (7.5)	13 (13.0)	
Fasting Glucose ≥ 110 mg/dL	No	344 (99.1)	134 (99.3)	280 (100.0)	100 (100.0)	0.388
	Yes	3 (0.9)	1 (0.7)	0 (0.0)	0 (0.0)	
BP ≥ 130/85	No	332 (95.7)	131 (97.0)	268 (95.7)	94 (94.0)	0.731
	Yes	15 (4.3)	4 (3.0)	12 (4.3)	6 (6.0)	

Abbreviations: BMI. Body Mass Index; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; HDL, High Density Lipoprotein; LDL, Low Density Lipoprotein; MetS, Metabolic Syndrome; BP, Blood Pressure.

Different superscript letters indicate significant differences among dietary patterns at $p < 0.05$ in the Tukey comparison.

[α] HOMA-IR = insulin (µU/ml) * glucose (mg/dL) /405 [48];
ǂ According the definition of NCEP ATP III - National Cholesterol Education Program - Adult Treatment Panel III [49];
[β] According to sex: waist circumference >102 cm for men and >88 cm for women;
[Ω] According to sex: <40 mg/dL for men and <50 mg/dL for women.