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Associations of diet and physical activity with body mass index, body fat and waist circumference in Azorean adolescents

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PALAVRAS-CHAVES: ADOLESCENTES; EXCESSO DE PESO/OBESIDADE; ALIMENTAÇÃO; PRODUTOS LÁCTEOS; ATIVIDADE FÍSICA.

To my parents, my brothers and Pedro

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Table of contents

List of figures

List of tables

Resumo

A elevada prevalência de obesidade infantil observada em países desenvolvidos e em vias de desenvolvimento é, segundo a Organização Mundial de Saúde, um grave problema de saúde pública. Embora a obesidade em crianças e adolescentes acarrete numerosas comorbilidades, o problema de saúde major será observado na próxima geração, na medida em que uma criança obesa tem maior probabilidade de se tornar um adulto obeso. Entre os fatores associados à obesidade, encontra-se descrito que a alimentação e a atividade física têm um papel fundamental no desenvolvimento desta condição. Entre os fatores alimentares, estudos recentes em crianças e adolescentes sugerem que o consumo de produtos lácteos possa ter um papel protetor contra a obesidade. Neste contexto, o objetivo deste trabalho foi determinar a associação entre o consumo de alimentos, particularmente de produtos lácteos, a atividade física e a ocorrência de valores excessivos de índice de massa corporal, perímetro da cintura e/ou percentagem de massa gorda, em adolescentes. Assim, realizou-se um estudo de base escolar, o "The Azorean Physical Activity and Health Study II", numa amostra representativa de 1515 adolescentes (dos 15 aos 18 anos) da Região Autónoma dos Açores. Foram recolhidos dados relativos ao peso, altura, perímetro da cintura e percentagem de massa gorda medidos segundo protocolos s*tandard* e instrumentos adequados. Dados relativos a características sociodemográficas e de estilo de vida foram obtidos através de um questionário. A ingestão alimentar e nutricional foi avaliada por questionário de frequência de consumo alimentar semi-quantitativo autoadministrado. Utilizou-se o questionário de Telama et al. (1997) para se recolher informação sobre a atividade física. A normalidade das variáveis foi avaliada através do teste estatístico de Kolmogorov-Smirnov. Para avaliar a existência de diferenças com significado estatístico foi utilizado os testes *t* ou Mann-Whitney e os testes de *One-Way Anova* ou Kruskal-Wallis para as variáveis contínuas. O teste do qui-quadrado foi utilizado para as variáveis categóricas. Foram, ainda, utilizados modelos de regressão (logística e linear múltipla) para estimar a magnitude da associação entre a ingestão de alimentos, atividade física e as componentes de obesidade. Assim, destacam-se como principais resultados: maiores consumos de leite e cereais *prontos-a-comer*, bem como melhores características de emprego do pai parecem ter uma associação inversa com a obesidade abdominal; os níveis de atividade física são menores em adolescentes com obesidade abdominal; o consumo de leite parece estar mais inversamente associado com as componentes de excesso de peso/obesidade do que o iogurte e o queijo; e o consumo de leite parece ter uma associação negativa com a ocorrência de obesidade abdominal independentemente da atividade física.

PALAVRAS-CHAVES: ADOLESCENTES; EXCESSO DE PESO/OBESIDADE; ALIMENTAÇÃO; PRODUTOS LÁCTEOS; ATIVIDADE FÍSICA.

Abstract

According to the World Health Organization, the high prevalence of childhood obesity is a serious public health problem that can be observed in developed and developing countries alike. Although obesity in children and adolescents entails numerous morbidities, the major health problem will be seen in the next generation, since an obese child is most likely to be an obese adult. Among the factors associated with obesity, it is described that diet and physical activity play key roles in the development of this condition. Concerning diet, recent studies suggest that the consumption of dairy products may protect against obesity. In this context, the objective of this study was to determine the association between diet, particularly the intake of dairy products, physical activity and the occurrence of excessive body mass index, waist circumference and/or percent body fat in adolescents. Thus, we carried out a school-based study, "The Azorean Physical Activity and Health Study II," with a representative sample of 1,515 Azorean adolescents (15 to 18 years old). Anthropometric measurements, such as weight, height, waist circumference and percent body fat, were collected according to standard protocols. Data on sociodemographic and lifestyle features were assessed by a questionnaire. Dietary and nutritional intake were measured via a self-administered semi-quantitative food frequency questionnaire. Physical activity was assessed via a self-report questionnaire by Telama et al. (1997). The Kolmogorov–Smirnov test was used to assess the assumption of normality. Independent sample *t* or Mann-Whitney tests and one-way analysis of variance or Kruskal-Wallis tests were performed to compare continuous variables. The Chi-square test was used with categorical variables. We also used regression models (multiple linear and logistic) to estimate the association between diet, physical activity and components of obesity. Thus, we highlight as our main results: higher intake of milk and ready-to-eat cereals, as well as father's employment status, seem to have an inverse association with abdominal obesity; physical activity levels are lower in adolescents with abdominal obesity; milk intake seems more likely to be inversely associated with components of obesity than do yogurt and cheese intake; and high milk intake seems to have a protective effect against abdominal obesity, regardless of physical activity level.

KEYWORDS: ADOLESCENTS; OVERWEIGHT/OBESITY; DIET; DAIRY PRODUCTS; PHYSICAL ACTIVITY.

List of abbreviations

$1,25(OH)_2D_3$	1,25-dihydroxyvitamin D_3
11-*β*-HSD-1	11-*β*-hidroxysteroid dehydrogenase type 1
BIA	bioelectrical impedance analysis
BMI	body mass index
%BF	percent body fat
CCK	cholecystokinin
CLA	conjugated linoleic acid
CT	computerized tomography
DXA	dual energy x-ray absorptiometry
DONALD	DOrtmund Nutritional and Anthropometric Longitudinally Designed
GH	growth hormone
GLP-1	glucagon-like peptide-1
HBSC	Health Behaviour in School-Aged Children
HELENA	Healthy Lifestyle in Europe by Nutrition in Adolescence
IGF	Insulin-like growth factors
MRI	magnetic resonance imaging
NHANES	National Health and Nutrition Examination Survey
PPAR-γ	peroxisome proliferator-activated receptor-gamma
PYY	peptide tyrosine tyrosine
RTEC	ready-to-eat cereal
SES	socioeconomic status
SSB	sugar-sweetened beverage
UCP2	uncoupling protein-2

WC	waist circumference
Waist-to-height ratio	WHtR

General introduction

According to the World Health Organization, adolescence is the period in human life that occurs after childhood and before adulthood, from ages 10 to 19 (World Health Organization, 2012). It represents one of the critical transitions in the life span and is characterized by a tremendous increase in the pace of growth and development, as well as by numerous anatomical, physiological, psychological, emotional and social changes. Adolescence is also the critical time of life in which many lifestyle patterns are developed and may be passed through to adulthood (Lake et al., 2006; Steinberg, 2005; Telama et al., 1997). Adolescents also gain increased control over their own food choices and health behaviors, often acquiring poor dietary patterns and decreasing their participation in physical activities (Keast et al., 2010; Nader et al., 2008; Sallis et al., 2000). Evidence has been demonstrated that low physical activity and inadequate nutrition are related to the energy imbalance that is the primary cause of obesity.

Obesity in children and adolescents is a major public health problem, reaching alarming rates in developed and developing countries alike (World Health Organization, 2011). Surveys during the 1990s show that in Europe, an additional 0.7% of all school-aged children became obese each year (Wang & Lobstein, 2006). Recent data on the prevalence of overweight and obesity in Europe shows that approximately 25% of children and adolescents are overweight or obese (International Association for the Study of Obesity, 2011; Wang & Lobstein, 2006). Obesity in adolescence is associated with enhanced risk of several common complex diseases, such as type 2 diabetes, hypertension and cardiovascular disease, as well as adverse socioeconomic and psychosocial sequelae (Lobstein et al., 2004). Although obesity in children and adolescents brings a number of additional problems with it, the greatest health problems will be seen in the next generation of adults, as the present childhood obesity epidemic passes through to adulthood (Lobstein et al., 2004). In this context, scientific investigation has focused on understanding the role of diet in the development of obesity in children and adolescents. Evidence suggests that dietary patterns characterized by high intake of sugar-sweetened beverages (SSBs) and foods with high energy density and low nutritional content (i.e., fast-food, 'junk' and snack food) may be associated with excess body weight and body fat (Agostoni et al., 2011; Newby, 2007). Regarding the independent effects of major food groups, such as fruits, vegetables and cereals, studies are inconsistent, making it difficult to draw conclusions about the association of these groups with obesity (Newby, 2007). In the last decade, increasing attention has been focused on the preventive effects of dairy product intake on obesity, and evidence in children and adolescents has suggested a beneficial or neutral effect of dairy food consumption (Spence et al., 2011). Although calcium has been mentioned as a principal bioactive component in explanations of how dairy product intake influences fatness, with effects on fat absorption and adipocyte lipid metabolism, other constituents, such as proteins (in particular, whey proteins) and their peptide derivatives may affect body fat by regulating appetite and food intake (Dougkas et al., 2011).

As seen above, physical activity is another possible factor in the development of obesity. Indeed, evidence suggests that obese adolescents are less physically active than non-obese adolescents and spend more time in sedentary pursuits, such as watching television and using other electronic media (Hills et al., 2011). Furthermore, it is widely recognized that physical activity is essential for the normal growth and development of children and adolescents.

In light of considerations that emphasize the increased prevalence of obesity in adolescents, changes in their eating habits and physical activity, the main objective of this thesis was to determine the relationship between diet and physical activity on measures of obesity (defined by excessive body mass index - BMI, waist circumference – WC, and/or percent body fat - %BF), focusing particularly on the possible relationships between dairy product intake and obesity in adolescence.

To reach this general objective, specific goals were drawn which led to original papers:

1. to investigate the association between dietary intake, physical activity and socioeconomic factors and obesity in adolescents:

   Paper I – Abreu, S., Santos, R., Moreira, C., Santos, P. C., Mota, J., & Moreira, P. Food consumption, physical activity and socioeconomic factors related to body mass index, waist circumference and waist-to-height ratio among adolescents. [submitted – under review]

2. to assess the association between dairy product intake and obesity among adolescents:

   Paper II - Abreu, S., Santos, R., Moreira, C., Vale, S., Santos, P. C., Soares-Miranda, L., Marques, A. I., Mota, J., & Moreira, P. (2012). Association between dairy product intake and abdominal obesity in Azorean adolescents. *Eur J Clin Nutr*, 66, 830–835.

   Paper III - Abreu, S., Santos, R., Moreira, C., Vale, S., Santos, P. C., Soares-Miranda, L., Mota, J., & Moreira, P. Milk intake is inversely related to body mass index and body fat in girls. (2012). *Eur J Pediatr,* May 1. [Epub ahead of print]

3. to identify the association of dairy intake and physical activity on obesity in adolescents:

   Paper IV - Abreu, S., Santos, R., Moreira, C., Vale, S., Santos, P. C., Soares-Miranda, L., Autran R., Mota, J., & Moreira, P. (2012). Relationship of milk intake and physical activity to abdominal obesity among adolescents. [submitted – under review]

Thus, the present dissertation is organized into four chapters. Chapter 1 provides a broad theoretical background to obesity. Chapter 2 is the experimental work, which includes the papers published or submitted to peer-reviewed scientific journals. An overall discussion is presented in Chapter 3, and in Chapter 4, the main conclusions of the present thesis and perspectives for future research are explained.

Moreover, in view of the fact that most studies include a wide age range and often use the term 'children' to define children under the age of 18, and because studies that include exclusively adolescents are scarce, in this dissertation, it has not been possible to present studies that only include adolescents. Therefore, this dissertation presents evidence from both children and adolescent studies.

Chapter 1

Theoretical background

Assessment of obesity

A simple definition of obesity is an excess of body fat, while overweight is an excess of body mass. Therefore, the underlying assumption of using different methods to define obesity is that a given measure is associated with fatness. It is noteworthy that the primary purpose of defining overweight and obesity is to predict health risks and provide comparisons between populations (Lobstein et al., 2004). Hence, the measurement of fatness in children and adolescents occurs in a range of settings, using a range of methods.

BMI, calculated from weight and height [weight (kg)/height^2 (m)], is commonly used in epidemiological studies as an indicator of overweight and obesity. BMI is significantly associated with relative fatness in childhood and adolescence and is the most convenient way of indirectly measuring body fat (Bellizzi & Dietz, 1999; Lobstein et al., 2004). Not surprisingly, because body weight is also correlated with muscle and lean mass, BMI tends to be correlated with muscle and lean mass, as well, and may be correlated with height within age groupings (Flegal & Ogden, 2011). Thus, two people with the same amount of body fat can have quite different BMIs (Lobstein et al., 2004). Yet, international definitions of overweight and obesity for children and adolescents are based on BMI, which is specific to age and gender (Cole et al., 2000; Must et al., 1991).

WC has been used as an anthropometric measure of abdominal obesity and is strongly associated with visceral adipose tissue (de Koning et al., 2007; Taylor et al., 2000). Furthermore, abdominal obesity is an independent risk factor for insulin resistance, hyperinsulinemia, dyslipidemia and hypertension in youth (Bacha et al., 2003; Wajchenberg, 2000). In children and adolescents, a WC equal to or above the 90th percentile is an independent risk factor for diseases related to abdominal obesity (Zimmet et al., 2007). The waist-to-height ratio (WHtR) is another measure used to assess abdominal obesity and the health risks associated with it, the underlying rationale for this ratio being that for a given height, there is an acceptable degree of fat stored in the upper body (McCarthy & Ashwell, 2006). WHtR is more closely linked to cardiometabolic risk factors than is BMI in children and adolescents (Hara et al., 2002; Savva et al., 2000); in adults, WHtR shows superiority over WC and BMI in the detection of cardiometabolic risk factors (Ashwell et al., 2012).

Other indirect methods of estimating body fat include the measurement of weight-for-height and skinfold thickness. Weight-for-height measurements have become a common means of assessing populations of children aged less than 10, but the measure is not useful in older children (Cole T & Rolland-Cachera MF, 2002). Skinfold thickness uses simple equipment and has the ability to determine total body fat and regional fat distribution. Yet, in very obese individuals, measurement of skinfold thickness may not be possible, and the relationship with metabolic problems is unclear (Lobstein et al., 2004).

Bioelectrical impedance analysis (BIA) is not strictly a direct measure of body composition, being based on the principle that, relative to water, lean tissue has a higher electrical conductivity and lower impedance than fatty tissue because of its electrolyte content. BIA has been shown to be better correlated with body fat than BMI or weight-for-height index (Tyrrell et al., 2001).

More accurate and precise methods are available to directly measure total body fat, including underwater weighing, magnetic resonance imaging (MRI), computerized tomography (CT), dual energy x-ray absorptiometry (DXA) and air-displacement plethysmography, but these cannot be feasibly applied in large epidemiological studies because they are complex, time-consuming and expensive. On the other hand, such methods are used predominantly for research and in tertiary care settings, and as a ‘gold standard’ to validate indirect measures of body fatness.

Obesity prevalence

Obesity in childhood and adolescence is one of the major public health problems of this century (World Health Organization, 2011). The prevalence of obesity has reached alarming rates in developed and developing countries alike

(World Health Organization, 2011). Data from the 1970s to the end of the 1990s show that the prevalence of overweight and obesity in school-aged children doubled or tripled in several regions, including North (Canada and the United States) and South America (Brazil and Chile), the Western Pacific region (Australia and China) and Europe (Spain, Greece, Finland, Germany and the United Kingdom) (Wang & Lobstein, 2006). Globally, 200 million school-aged children are classified as either overweight or obese, with 40-50 million obese (International Association for the Study of Obesity & International Obesity Taskforce, 2010). In the European Union, the prevalence of children and adolescents who are overweight or obese is approximately 25%, with Southern European countries showing the highest prevalence rates (International Association for the Study of Obesity, 2011; Wang & Lobstein, 2006). A recent survey in Portugal found that 21.6% of girls and 23.5% of boys aged 10 to 18 are overweight or obese (Sardinha et al., 2011). In Spain, the prevalence was 22.9% in girls and 32.9% in boys aged 5 to 17 (Serra Majem et al., 2003), while data from Greece show that 37.0% of girls and 45.0% of boys aged 10 to 12 to be overweight or obese (Manios et al., 2011). Northern European countries tend to have lower prevalence; for instance, in Norway 14.7% of girls and 12.9% of boys were overweight (Juliusson et al., 2007). Overall, children in northern Europe generally have overweight/obesity prevalence rates of 10–20%, while, in southern Europe, the prevalence rates are 20–35% (International Association for the Study of Obesity, 2011).

Complications of obesity

Adolescence is seen as a period of life that involves rapid physical growth and dramatic psychosocial change (Steinbeck, 2009). Health-damaging behavior during adolescence can lead to health problems, both immediately and in the years ahead. As seen above, obesity has become one of the most prevalent nutritional concerns in adolescents and children and has been clearly linked to physical and psychosocial comorbidity. According to the evidence, childhood obesity is likely to persist into adulthood, and obese children are more likely to develop noncommunicable diseases, like type 2 diabetes, hypertension and cardiovascular disease, at a younger age, making this generation the first predicted to have a shorter lifespan than their parents (Guo et al., 2002; Lobstein et al., 2004). In addition to increased future risks, obese children and adolescents can experience breathing difficulties (such as sleep-disordered breathing and asthma), increased risk of fractures, insulin resistance, psychological and social consequences (i.e., low self-esteem and poor self-concept, reduced quality of life, depression and social discrimination) (Lobstein et al., 2004). It has also been reported that excess body weight affects endocrine response, with early menarche in girls and delayed maturation in boys (Freedman et al., 2003; Wang, 2002). Moreover, resources applied to the management of obesity and the conditions associated with or caused by obesity have an impact on health care costs. In developed countries, studies suggest that obesity accounts for 2 to 7% of total health care costs (World Health Organization, 2000). In Portugal, it is estimated that complications associated with obesity are responsible for 5 to 10% of health costs (Pereira & Mateus, 2003).

Etiology of obesity

Many studies have focused on the genetic, molecular and cellular aspects of weight and fat gain and the various physical and environmental risk factors that increase the likelihood that a child will be obese (Lobstein et al., 2004). Although there are clearly strong genetic influences on susceptibility to obesity, large changes in its prevalence over such a short time must reflect major changes in nongenetic factors, providing tacit evidence that some instances or aspects of obesity must be responsive to, or preventable by, manipulation of the environment (Rosenbaum & Leibel, 1998).

A number of studies have shown that maternal weight gain and diabetes during pregnancy, as well as birth weight, are positively related to subsequent fatness, suggesting that the fetal environment plays a role in the development of obesity (Gillman et al., 2003; Oken, 2009; Schack-Nielsen et al., 2010; Seidman et al., 1991; Whitaker & Dietz, 1998; Yu et al., 2011). It is noteworthy that the association between birth weight and risk of obesity is not linear, being reported as a U-shaped relationship with a higher prevalence of obesity during childhood and adulthood, seen in both the lowest and highest birth weights (Yu et al., 2011). After birth, method of feeding may also influence the development of obesity. Several studies suggest that breastfeeding protects against obesity during childhood and adolescence (Armstrong & Reilly, 2002; Gillman et al., 2001; McCrory & Layte, 2012; von Kries et al., 1999; Weyermann et al., 2006), but others argue that the apparent effect may be due to confounding factors, such as socioeconomic status (SES), maternal smoking, maternal fatness, maternal diabetic status or infant birth weight (Hediger et al., 2001; Owen et al., 2005; Poulton & Williams, 2001).

The identification of growth periods critical to the development of childhood and adolescent obesity has sparked scientific curiosity. During growth, children experience a rapid increase in BMI during the first year of life and a decline after that (around 9 to 12 months), with BMI reaching a minimum, on average, from age 5 to 7. The point of maximal leanness or minimal BMI has been called the adiposity rebound, and several studies have shown that earlier rebound is associated with a subsequent increased risk of overweight and obesity (Rolland-Cachera et al., 1984; Rolland-Cachera et al., 2006; Whitaker et al., 1998).

Obesity in children and adolescents is also linked to parental obesity, likely owing to a combination of genetic, social and environmental factors (Lake et al., 1997; Lobstein et al., 2004). Children with two obese parents have a higher risk of becoming obese than those with one or no obese parents (Lake et al., 1997).

SES has long been considered a factor related to the development of obesity (Brisbois et al., 2012). In developed countries, low SES is more likely to be associated with increased obesity prevalence than is high SES (Brisbois et al., 2012). Some studies have also described other factors as proxy measures for SES, such as parents' employment. As shown recently, father's employment status, in contrast to mother's employment status, showed a strong and consistent relationship with increased risk of children's developing obesity. It seems that lower employment status of the father is associated with the adult obesity of offspring (Brisbois et al., 2012).

Despite the effects of genetic and non-genetic factors, such as those mentioned above, the key determinant of overweight and obesity is energy imbalance - a disruption of the balance between energy intake and energy expended. Thus, the steady increase of overweight/obese children and adolescents in recent decades may be the result of an increase in energy intake, a decrease in energy expenditure or, more logically, a combination of both (Hills et al., 2011). Although physiological systems (endocrine, gastrointestinal, central and peripheral nervous, and cardiovascular) and behavioral factors affect this equation, according to evidence, diet and physical activity are two essential determinants. These two factors will be dealt with in more detail subsequently.

Food intake regulation

In the context of the high prevalence of obesity, it is important to consider the impact of food intake regulation on energy balance and whether it can be enhanced, in order to facilitate the reduction of energy intake.

Regulation of food intake is controlled by several regulatory systems that produce a variety of signals, such as appetite, satiation and satiety. The peripheral and central nervous systems are involved in short-term and long-term regulation of food intake, acting synergistically to either stimulate or suppress food intake (Dougkas et al., 2011; Schwartz et al., 2000).

In the short term, food-modulated regulatory signals from the gastrointestinal tract, along with signals generated from dietary factors before and during food metabolism, inform the central nervous system of food intake and regulate satiation (i.e., processes that promote meal termination, thereby limiting meal size) and satiety (i.e., a postprandial event that affects the interval to the next meal, thereby regulating meal frequency) (Cummings & Overduin, 2007; Lobstein et al., 2004). Peptides found in the enteric nervous system and entero-endocrine cells of the gastrointestinal tract are involved in short-term regulation of food intake. It is in the gastrointestinal tract that a variety of satiety signals that act mainly through the brainstem are initiated. The gut peptide hormones and other bioactive peptides involved in short-term regulation of appetite and food intake are listed in Table 1. Insulin and leptin, although minor short-term regulators of food intake, are also involved in this pathway, increasing the actions of peripheral satiation signals via cholecystokinin (CCK) (Morton et al., 2006). It is noteworthy that the secretion and regulation of these peptides depend on both the macronutrient composition of the diet and neuroendocrine factors. On the other hand, there is evidence that satiety is also influenced by learned habits (Strubbe & Woods, 2004).

In the longer term, a series of homeostatic mechanisms are involved in regulating fat storage and release, resting metabolic rate and energy expenditure, which may be largely determined by genetic mechanisms and susceptible to genetic variation (Lobstein et al., 2004). The adipocyte-derived leptin, pancreatic insulin and ghrelin are the major hormones involved in long-term regulation of appetite. Leptin and insulin regulate food intake by increasing the secretion of anorexigenic (appetite-suppressing) neuropeptides and decreasing the secretion of orexigenic (appetite-stimulating) neuropeptides, while ghrelin has the opposite effects (Dougkas et al., 2011; Schwartz et al., 2000). The secretion of leptin and insulin is highly correlated with adiposity and, consequently, individuals who are obese have high leptin and insulin levels, but the traditional effects of these hormones on the central nervous system - decreasing appetite and increasing energy expenditure - become inefficient, and the individual continues to consume food, despite having a positive energy balance (Golub et al., 2011). This decreased responsiveness is also known as leptin and insulin resistance. The actions of hormones and neuropeptides involved in long-term regulation of food intake are listed in Table 1.

Table 1 - Hormones and neuropeptides involved in regulation of food intake (adapted from Dougkas et al., (2011)).

	Main site of synthesis	Effect on food intake
Short-term		
Cholecystokinin	Proximal intestinal L cells	↓
Glucagon-like peptide-1	Distal intestinal L cells	↓
Peptide tyrosine tyrosine	GI tract	↓
Oxyntomodulin	Distal-intestinal L cells	↓
Bombesin (gastrin-release peptide and neuromedin B)	Stomach	↓
Opioids	Dietary BAP	↓
Leptin	Adipose tissue	↓
Insulin	β cells of pancreas	↓
Ghrelin	Stomach	↑
Long-term		
Leptin	Adipose tissue	↓
Insulin	β cells of pancreas	↓
Pro-opiomelanocortin	Hypothalamus	↓
α-Melanocyte-stimulating hormone	Hypothalamus	↓
Corticotrophin-releasing hormone	Hypothalamus	↓
Thyrotropin-releasing hormone	Hypothalamus	↓
Ghrelin	Stomach	↑
Neuropeptide Y	Hypothalamus	↑
Agoutini-related protein	Hypothalamus	↑
Melanin-concentrating hormone	Hypothalamus	↑
Orexins	Hypothalamus	↑

BAP, bioactive peptides; GI, gastrointestinal; ↑, stimulation; ↓, suppression.

Physical activity

It is documented that physical activity gradually declines in the period of transition from childhood to adolescence, between the ages of 9 and 15 (Nader et al., 2008; Sallis et al., 2000); physical activity becomes less of a priority and is displaced by other behaviors (Hills et al., 2007). In addition, there is evidence that many children and adolescents participate in considerably less physical activity than is recommended for the maintenance of optimal health (Reilly et al., 2004; Riddoch et al., 2004; Riddoch et al., 2007).

Most investigations of the role of physical activity in the development of obesity have been cross-sectional. Although the literature shows inconsistent results, several studies have reported that overweight/obese children and adolescents are less active than their lean counterparts (Page et al., 2005; Planinsec & Matejek, 2004; Trost et al., 2001). The evidence highlights that low levels of physical activity may be both an antecedent to and a consequence of weight status. In adolescence, when physical activity often occurs as part of school classes, overweight/obese adolescents may be less likely to want to participate, either due to fears of teasing or being ostracized, or because they are 'less athletic' (Must & Tybor, 2005).

The evidence available from prospective studies suggests that increased physical activity protects against relative weight and fatness gains over childhood and adolescence (Berkey et al., 2000; Gordon-Larsen et al., 2000). Data from the Growing Up Today Study, with followed adolescents for 1 year, showed that physical activity was inversely related to change in self-reported BMI in girls, whereas similar results in boys were of borderline significance (Berkey et al., 2000). Follow-up data from a nationally representative sample of 12,759 US youth in the National Longitudinal Study of Adolescent Health found that each additional bout of moderate-to-vigorous activity per week was associated with lower odds of becoming overweight (Gordon-Larsen et al., 2000). In addition, the Amsterdam Growth and Health Longitudinal study demonstrated that physical activity in 13-year-old adolescents was inversely related to fat mass over the next 20 years, as measured longitudinally by the sum of four skinfolds (Kemper et al., 1999). The authors of the latter concluded that promotion of habitual physical activity during adolescence seems to be an effective means of preventing obesity.

Whereas increased physical activity protects against overweight/obesity during childhood and adolescence, increased sedentary behavior is positively related to obesity. Time spent on sedentary behaviors like watching television, playing video or computer games and using the Internet have been individually associated with increased body weight and/or adiposity in cross-sectional (te Velde et al., 2007; Vandelanotte et al., 2009) and prospective studies (Mitchell et al., 2012). It has been proposed that sedentary and active behaviors can co-exist in the same individual and that one type of behavior does not automatically displace the other (Biddle et al., 2004). However, sedentary behavior must be limited because of its contribution to positive energy balance and its association with overeating (Hills et al., 2007).

It has been observed that physical activity can influence the regulation of food intake by either adjusting the sensitivity of appetite control mechanisms or by generating an energy deficit that can adjust energy intake (Blundell & King, 1999). According to a review of the literature from doubly-labeled water studies, an exercise-induced increase in energy requirement is typically compensated for by increased energy intake, while a change from a physically active to a more sedentary routine may not induce an equivalent reduction of energy intake and generally results in weight gain (Westerterp, 2010). Although the impact of exercise on energy intake is still controversial, it has been reported that medium- to long-term exercise improves satiety response to meals and the sensitivity of the appetite control system (Chanoine et al., 2008; Martins et al., 2010).

Beyond the protective effects of physical activity on obesity, physical activity is also important to the physical, mental and social aspects of growth and development, helping to set a pattern of participation in physical activity across the lifespan (Hills et al., 2007).

Energy intake, energy density and nutrients

The study of the relationship between diet and health began by examining the role that specific nutrients can have on the etiopathogenesis of such diseases as obesity (Roman-Vinas et al., 2009). Indeed, food composition affects energy balance, due to its influence on satiety, satiation and hunger, as well as substrate utilization (Rodriguez & Moreno, 2006). Although energy intake has a clear impact on energy balance, data on the relationship between total energy intake and obesity among children and adolescents is conflicting (Newby, 2007). A former review of childhood obesity indicates that obese children do not tend to have higher total energy intake (Schonfeld-Warden & Warden, 1997), which supports findings from cross-sectional studies that show mean energy intake does not significantly differ among overweight and normal weight children (Ball et al., 2005; Kelishadi et al., 2003). Evidence for a lack of association also comes from well-designed prospective studies (Jago et al., 2005; Magarey et al., 2001). However, higher total energy intake was observed in a few cross-sectional (Azizi et al., 2001; Gillis et al., 2002) and prospective studies (Berkey et al., 2000) among overweight and obese children. The discrepancies in the findings of existing studies may be due to inadequate control of potential confounders, differences in methods of assessing diet and body composition and lack of consideration of the role of underreporting (Newby, 2007).

Energy density is an important aspect of energy intake and has received increasing attention in relation to obesity. Energy density is defined as the amount of energy available per unit of weight of food or beverage (kcal/g or kJ/g). It is highest in foods containing high levels of fat and/or significant amounts of refined carbohydrates, such as sugar, and can also be viewed as an inverse of nutrient density (Lobstein et al., 2004). In children and adolescents, few studies have evaluated the relationship between energy density and obesity. According to the findings of cross-sectional (Vernarelli et al., 2011) and prospective studies (Johnson et al., 2008), energy-dense diets are positively associated with overweight and/or obesity. Some studies, however, have found a null association (Kral et al., 2007; McCaffrey et al., 2008). Although there are no reports of negative associations, the contribution of energy density to childhood obesity remains unclear (Wilks et al., 2011).

The role of macronutrients (as a total or relative percentage of energy intake) is another issue considered in relation to the etiology of obesity. Evidence suggests that macronutrients generate different signals of satiety. Fat, for instance, has a weaker effect on satiety than protein, which has been found to be the most satiating, while carbohydrates have an intermediate effect (Stubbs et al., 1996). In addition, sugar- and fat-rich foods are very palatable and flavorful, increasing the likelihood of greater energy intake due to passive overconsumption (Newby, 2007). In this line, several cross-sectional studies have reported a positive relationship between the proportion of total energy intake covered by fat and body fatness (Maillard et al., 2000; McGloin et al., 2002; Tucker et al., 1997), whereas inverse associations have been shown with carbohydrate intake in children and adolescents (Tucker et al., 1997). In contrast, a number of prospective studies have not been able to establish any association between self-reported dietary fat or carbohydrate intake and subsequent weight and body fat change (Berkey et al., 2000; Magarey et al., 2001; Newby et al., 2003).

The effect of type of fat on obesity has received relatively little attention. Cross-sectional studies have reported that obese children have higher saturated fat intake than their lean counterparts (Gazzaniga & Burns, 1993; Gillis et al., 2002). One prospective study observed a significant inverse association between monounsaturated fat and %BF, although the findings were not robust across several models (Carruth & Skinner, 2001). Concerning polyunsaturated fatty acids, animal and human studies have shown that n-3 long chain fatty acid supplementation, specifically eicosapentaenoic acid and docosahexaenoic acid, may protect against obesity and may reduce weight gain in already obese animals and humans (Buckley & Howe, 2009; Golub et al., 2011). Experimental studies in adults have reported that a diet rich in fish oil increases basal fat oxidation (Couet et al., 1997) and reduces fat mass (Noreen et al., 2010). Accordingly, randomized controlled trials have reported significant weight loss when n-3 long chain polyunsaturated fatty acids are administered together with an energy-restricted diet (Krebs et al., 2006; Kunesova et al., 2006). In children and adolescents, although studies are scarce, it has been suggested that obese children have lower profiles of n-3 long chain polyunsaturated fatty acid plasmas than their lean counterparts (Burrows et al., 2011; Scaglioni et al., 2006). Furthermore, recent prospective (Donahue et al., 2011) and randomized controlled studies (Lucia Bergmann et al., 2007) have suggested that n-3 long chain polyunsaturated fatty acid intake during pregnancy and lactation may affect the risk of obesity in offspring. Regarding the effects of other fatty acids, such as *trans*-fatty acids, which are derived mainly from the partial hydrogenation of vegetable oils, evidence from epidemiological studies is limited but consistent and shows that increased consumption of *trans*-fatty acids may result in small additional weight gain (Thompson et al., 2011). The potential role of conjugated linoleic acid (CLA) on body fat reduction has also been explored, and, as recently reported in a randomized controlled study, supplementation of CLA, seems to attenuate significantly body fat deposition in overweight and obese prepubertal children (Racine et al., 2010). However, the results from humans studies are inconsistent, and long-term investigation evaluating the safety and efficacy of CLA supplementation in pediatric populations is needed (Agostoni et al., 2011; Racine et al., 2010). Thus, the relevance of different types of fat to childhood obesity requires further investigation.

The role of protein intake in childhood obesity has been reported in few studies. In two cross-sectional studies, mean intake of protein (as a percentage of energy) was significantly higher in overweight and obese adolescents than their lean counterpart (Lima et al., 2004; Ortega et al., 1995), whereas, in another study, no significant differences were seen between children with high and low BMI Z-scores (Elliott et al., 2011). Accordingly, prospective studies have found no relationship between protein intake and weight or body fat (Ball et al., 2005; Magarey et al., 2001). In addition, some studies have reported that an association between high protein intake in early childhood and a relatively early ‘adiposity rebound’ may increase the risk of being obese in childhood and adolescence (Lobstein et al., 2004; Rolland-Cachera et al., 1995; Rolland-Cachera et al., 2006). Data from the German DOrtmund Nutritional and Anthropometric Longitudinally Designed (DONALD) study suggest that high protein intake at the age of 12 and between the age of 18-24 months was independently related to higher mean BMI standard deviation score and %BF at the age of 7 (Gunther et al., 2007). Moreover, emerging research suggests that the form of protein may be as important as the amount of protein in infant feeding. A recent clinical trial with children aged less than 4 months who were fed isocaloric formulas showed that infants whose formulas had higher free glutamate content had lower formula intake than infants who consumed formula lower in free glutamate (Ventura et al., 2012). The author of the study has stated that free glutamate may promote satiation and satiety during formula meals (Ventura et al., 2012).

There is also some evidence that degree of satiety is related to the glycaemic index of carbohydrates in food (Astrup, 1999). It has been postulated that a low-glycaemic diet decreases blood glucose and insulin excursion, promotes greater fat oxidation, decreases lipogenesis and increases satiety (Kong et al., 2011). A randomized controlled trial conducted with obese adolescents showed that after a 6-month intervention, BMI decreased significantly more in the group with the low-glycaemic diet, compared to the group with the conventional diet (reduced-fat) (Ebbeling et al., 2003). However, more studies are needed to establish the association between glycaemic index and obesity with greater certainty.

It has been suggested that dietary fiber intake may reduce the risk of developing obesity. Evidence for the hypothesized association between amount of dietary fiber and obesity risk is based upon ecological observations of low rates of obesity among populations with high dietary fiber intake and high rates of obesity among Western populations with low fiber intakes (Kimm, 1995; Newby, 2007). Dietary fiber contributes limited energy to the diet and also seems to prolong satiety by modulating the time course of pre- and post-absorptive satiety signals (Astrup, 1999). A review of randomized trials has concluded that the majority of studies show that high intake of dietary fiber promotes weight loss (Pereira & Ludwig, 2001).

Recently, the role of calcium and vitamin D has raised interest in obesity research. It has been reported that low calcium intake and vitamin D insufficiency often coexist with obesity (Soares et al., 2011). Although very few studies of children and adolescents have specifically examined the association between vitamin D and obesity, two cross-sectional studies have found an inverse relationship (Alemzadeh et al., 2008; Gordon et al., 2004). On the other hand, Foo et al. (2009) have found that vitamin D status, as assessed by plasma 25-hydroxyvitamin D concentration, which reflects supply of vitamin D from both the diet and from cutaneous synthesis under the influence of solar ultraviolet light (Hollis, 1996), was positively associated with lean body mass, but no association was found with degree of body adiposity. In a prospective study with children ages 5 to 12, even after controlling for baseline adiposity and other confounders, lower vitamin D status was associated with greater increases in BMI and WC (Gilbert-Diamond et al., 2010). The role of vitamin D in body weight and body fat requires, however, further well-designed studies of children and adolescents.

The role of calcium in obesity risk is described in several observational and experimental studies. Novotny et al. (2004), in a cross sectional study with girls aged 9 to 14, found that total calcium intake (i.e., from diet and supplementation) was negatively associated with both iliac skinfold thickness and weight ($\beta = -0.0025$, $P = 0.01$ and $\beta = -0.0027$, $P = 0.09$, respectively). Likewise, Moreira et al. (2005), in a large sample of Portuguese children, showed that calcium-to-protein ratio was inversely related to BMI only in girls ($\beta = -0.052$, $P = 0.002$). Other cross-sectional studies have yielded similar results (dos Santos et al., 2008; Goldberg et al., 2009; Palacios et al., 2007). To determine whether calcium intake is related to children´s body fat over time, Skinner et al. (2003) conducted a prospective study with young children between the ages of 2 months and 8 years. The latter indicated that dietary calcium intake was negatively related to %BF over time, was positively related to dietary variety and was negatively related to intake of carbonated drinks and other sweetened beverages. The authors recommend that children be strongly encouraged to regularly include calcium-rich foods and beverages in their diets, specifically skim, 1% or 2% fat milk and other low-fat dairy products. While the above study indicated inverse associations between calcium intake and body weight and/or body fat, there are other prospective studies in which no such associations have been noted (Barr, 2007; Fisher et al., 2004). Barr (2007) conducted an investigation to determine whether habitual dietary calcium intake was an independent predictor of %BF over 2 years of follow-up and found that there was no relation between calcium intake and changes in body composition among peripubertal girls. In a cohort of 192 girls, calcium intake was evaluated from ages 5 to 9, as a function of mother-daughter beverage choices and as a predictor of bone mineral status (Fisher et al., 2004). Girls who met the recommended adequate intake for calcium were not heavier than those who consumed less than the recommended adequate intake.

Like studies with children and adolescents, studies with adults are inconsistent. However, a recent meta-analysis by Dougkas et al. (2011) showed a significant inverse relationship between dietary calcium intake and BMI, which indicated that an 800 mg per day increase in calcium intake could reduce BMI by 1.1 kg/m^2.

Although the literature highlight the role of macro- and micronutrients in the development of obesity, the study of the effect of single nutrients does not consider interactions between nutrients and the inherent complexity of diet. Moreover, adolescence is a period of life where some eating behaviors are seen more frequently than in other age-groups, including meal skipping, the consumption of away-from-home foods and fast foods, and the choice of less healthy foods (e.g., high-fat snacks and SSBs) (Lytle et al., 2000; Moreno et al., 2010). Unhealthy eating habits acquired during childhood and adolescence may interfere with optimal growth and development while setting the stage for poor eating habits in adulthood (Kelder et al., 1994). Furthermore, adolescence is also the critical time when dietary habits are established (Cavadini et al., 2000).

Thus, a substantial body of evidence has emerged on the relationship between foods, beverages, dietary patterns, eating behaviors and obesity. The studies performed on these relationships in children and adolescents will now be discussed.

Food and beverage intake

Fruits and Vegetables

Fruit and vegetable intake is an important element of a healthy diet, providing essential nutrients and nutritive compounds (Yngve et al., 2005). There is only limited data on European children's fruit and vegetable consumption, but data from the Pro Children Project suggest that only 6-24% of European children consume more than the 400 g of fruit and vegetables recommended per day (Yngve et al., 2005). The highest intake of fruits and vegetables was seen in Portugal and Austria, both of which reached a mean intake of about 264 g per day (Yngve et al., 2005). In addition, recent data from the Health Behaviour in School-Aged Children (HBSC) study showed that Portugal is among the five countries with a higher proportion of adolescents aged 11 and 13 who reported eating fruit at least every day or more than once a day (Currie et al., 2012). It is noteworthy that the same study found that the prevalence of fruit consumption by both genders decreased between ages 11 and 15.

There is lack of evidence that high intake of fruits and vegetables is associated with lower risk of obesity in children and adolescents. Few cross-sectional studies among adolescents have observed an inverse association between the frequency of fruit and vegetable consumption and BMI (Kelishadi et al., 2003) and between fruit intake and body fat (Baric et al., 2001). Roseman et al. (2007) found that underweight and healthy-weight adolescents consumed more fruits than adolescents who were at risk of being overweight or were overweight. However, most cross-sectional studies have not found a protective association between combined or individual fruit and vegetable consumption and obesity in children and adolescents or have yielded findings that were inconsistent across age and sex groups (Colapinto et al., 2007; Matthews et al., 2011; Vagstrand et al., 2007; Violante et al., 2005). Few prospective studies have also examined the role of fruit and vegetable intake in obesity. Field et al. (2003), in a study with children and adolescents aged 9 to 14, reported that intake of fruits, fruit juices or vegetables (alone or combined) was not related to changes in BMI Z-score. Likewise, no significant association was seen in two other prospective studies between fruit and vegetable consumption and BMI among preschool children (Faith et al., 2006; Newby et al., 2003). Differences in findings may be due to wide variability in methods of cooking and preparing fruit and vegetables that contribute to differences in energy density and macronutrient composition, and which modify their effects on body weight (Matthews et al., 2011; Tohill et al., 2004).

Consumption of soup, as another means of vegetable intake, has been shown to be particularly effective in increasing satiety (Mattes, 2005) and has proved to be a promising approach to increasing vegetable intake (Spill et al., 2011). In a recent study with preschool-aged children, a serving of vegetable soup at the start of a meal resulted in a reduction of energy intake during the main course and of energy intake over the course of the entire meal (Spill et al., 2011). In addition to affecting energy intake, the serving of vegetable soup as a first course led to increased vegetable consumption. Bessa et al. (2008), in a cross-sectional analysis found, only in girls, that the odds of being overweight increased significantly for those having low consumption of vegetable soup (equal to or lower than the median - 342.9 g), compared to those with high consumption (higher than the median). This relationship has been better explored in adults, and the results from cross-sectional (Bertrais et al., 2001; Kuroda et al., 2011; Moreira & Padrao, 2006) and intervention studies (Jordan et al., 1981) suggest that soup intake is negatively correlated with obesity prevalence.

Ready-to-eat cereal

Most research on cereal has focused on ready-to-eat cereal (RTEC). In both children and adolescents, RTEC consumption is associated with improved diet quality, including the reduction of total cholesterol intake and an increase in dietary fiber and micronutrient intake (Kosti et al., 2010). Research findings have demonstrated that children and adolescents who consume cereal routinely are most likely to eat breakfast and least likely to be at risk for overweight and/or obesity (Albertson et al., 2003; Albertson, Thompson, et al., 2009; Barton et al., 2005; Kafatos et al., 2005; Kosti et al., 2008; Williams et al., 2009). In a cross-sectional study conducted on a sample of children aged 4 to 12, Albertson et al. (2003) showed that frequency of RTEC consumption was inversely associated with BMI within each age group, as well as for the total sample. Other cross-sectional studies have reported similar results (Kafatos et al., 2005; Kosti et al., 2008; Williams et al., 2009). For instance, Kafatos et al. (2005) found that RTEC consumption was inversely related to obesity indices in adolescents.

The relationship between RTEC consumption and weight status was examined in two prospective studies (Albertson, Thompson, et al., 2009; Barton et al., 2005). Data from the National Heart, Lung, and Blood Institute Growth Health Study showed that cereal consumption was predictive of lower BMI among black and white girls between the ages of 9 and 19 (Barton et al., 2005). Along these lines, another prospective study found that girls who ate cereal on a greater percentage of days during childhood had lower %BF and total cholesterol levels during study year 10 (Albertson, Thompson, et al., 2009). It is noteworthy that the findings of recently conducted prospective studies in children and adolescents have shown that an increase in RTEC intake is linked to lifestyle habits, such as high levels of physical activity and reduced television viewing (Albertson, Thompson, et al., 2009; Albertson et al., 2008), demonstrating that lifestyle issues might also have to be taken into consideration when explaining the inverse relationship between RTEC intake and BMI (Kosti et al., 2010).

Furthermore, results from randomized controlled trials are in accordance with cross-sectional and prospective studies. An intervention study with children aged 8 to 10 reported that more days of RTEC consumption were associated with lower BMI only in boys (Albertson, Affenito, et al., 2009). On the other hand, Rosado et al. (2008) showed that reducing obesity by increasing RTEC consumption was an effective strategy only when accompanied by nutritional education.

Thus, there is strong evidence that RTEC plays a protective role against childhood obesity. However, differences in the composition of RTEC must be considered in explanations of this 'antiobesity' effect (Kosti et al., 2010). For example, the total dietary fiber content of RTEC varies from 10 to 15%, while the soluble fiber content varies from 20% (wheat) to approximately 50% (oats) (Spiller GA, 1993). Moreover, RTEC are available in high- and low-glycemic index versions (Atkinson et al., 2008). Accordingly, the reported benefits of RTEC might be more pronounced among more nutrient-dense and fiber-rich cereals (Kosti et al., 2010).

'Junk Food' and Snack Food

Snacks can be defined as eating episodes, generally smaller and less structured than "meals" (Gatenby, 1997). More than 80% of adolescents reported eating snacks between meals that usually consisted of processed 'junk food' and tended to be low-nutrient and energy-dense foods (Jenkins & Horner, 2005). Such foods can displace more healthful 'snack' foods, such as fruits, vegetables and nuts (Newby, 2007). A cross-sectional study found that overweight Greek adolescents consumed more snacks (potato chips, chocolate bars, pizza, cheese pie and cream pie) than their non-overweight counterparts (Hassapidou et al., 2006). Likewise, another cross-sectional study of children showed that the prevalence of overweight was positively associated with snack food intake (of high-energy sweets and beverage) (McDonald et al., 2009). Consumption of fatty/salty snacks was also directly related to BMI among adolescents aged 11 to 18 (Kelishadi et al., 2003), while another study showed a positive association only among children with an overweight parent (Francis et al., 2003). On the other hand, Keast et al. (2010) showed that adolescents who engaged in more frequent snacking were less likely to be overweight or obese and less likely to have abdominal obesity.

Data from three prospective studies showed that low-nutritional-value snack foods were not an important independent determinant of weight gain among children and adolescents (Field et al., 2004; Francis et al., 2003; Phillips et al., 2004). However, one prospective study of preschool children reported a positive association between "fat food" (e.g., ice cream, potato chips, cookies, chocolate, fried foods) consumption and weight change (Newby et al., 2003). It has been suggested that the relationship between snack foods and risk of obesity may be mediated by genetics or the presence of an overweight parent or an environment that provides ubiquitous access to snack foods (Newby, 2007). Thus, more studies are needed to establish a relationship between snack foods and obesity.

Sugar-sweetened beverages

SSBs are defined as liquids that are sweetened with various forms of sugars that add energy, including, but not limited to, sodas, fruit ades and fruit drinks and sports and energy drinks (U.S. Department of Agriculture & U.S. Department of Health and Human Services, 2010). Recently, the 2010 Dietary Guidelines for Americans stated that there is strong evidence that children and adolescents who consume more SSBs have higher body weights than those who drink fewer SSBs, and moderate evidence also supports this relationship in adults (U.S. Department of Agriculture & U.S. Department of Health and Human Services, 2010).

Several cross-sectional studies have reported a positive association between SSB consumption and obesity among children and adolescents (Amin et al., 2008; Ariza et al., 2004; Giammattei et al., 2003; Novotny et al., 2004). Giammattei et al. (2003) found, in 11- to 13-year-old schoolchildren, that the mean BMI Z-score for those consuming less than 3 soft drinks per day was significantly lower than the same consuming 3 or more soft drinks per day. One study also reported that soda intake was significantly positively associated with weight in girls aged 9 to 14 (Novotny et al., 2004).

The findings of prospective studies are in accordance with cross-sectional results (Fiorito et al., 2009; Libuda et al., 2008; Ludwig et al., 2001; Phillips et al., 2004; Tam et al., 2006). One prospective study among children aged 11 to 12, with 19 months of follow-up, reported that for each additional serving of SSB consumed, both BMI and frequency of obesity increased, even after adjusting for anthropometric, demographic, dietary and lifestyle variables (Ludwig et al., 2001). In addition, another prospective study with a 21-year follow-up period showed that an increase in the consumption of SSBs from childhood to adulthood was associated with overweight in women (odd ratio = 1.90, 95% confidence interval: 1.38, 2.61), but not in men (Nissinen et al., 2009). However, not all cross-sectional (Valente et al., 2011), prospective (Johnson et al., 2007; Vanselow et al., 2009) and meta-analysis (Forshee et al., 2008) studies have shown a positive association between SSB consumption and BMI.

Among children and adolescents, most intervention studies are based on nutrition education that promotes a healthy diet and discourages or limits the consumption of SSBs. Nevertheless, only one study found, after 12 months of school-based intervention, that the prevalence of overweight was significantly higher in the control than in the intervention group (28.5% vs. 18.7%, respectively) (James et al., 2007). Three years after baseline, however, the differences were smaller and no longer significant.

Besides being related to obesity, regular SSB consumption has also been associated with increased energy intake (Vartanian et al., 2007), and metabolic syndrome and type 2 diabetes (Malik et al., 2010). Moreover, it has been described that SSB intake is associated with lower intake of milk, calcium and other nutrients and is related to other unhealthy lifestyle habits (Vartanian et al., 2007). The findings from the HBSC study have shown negative associations between SSB intake and breakfast consumption (Vereecken et al., 2009) and family rules, positive associations between frequent meal consumption in fast-food restaurants and high rates of television viewing associated to snacking and meal consumption (Currie et al., 2012; Verzeletti et al., 2010).

Fast foods and away-from-home foods

Fast foods and away-from-home foods may contribute to the obesity problem, as these foods tend to be higher in total fat, saturated fat, sugar and sodium, as well as lower in fiber, vitamins and minerals (French et al., 2001; Newby, 2007). In addition, these foods have high palatability (appealing to primordial taste preferences for fats, sugar and salt), and their portion sizes are large, which may also cause excessive weight gain (Agostoni et al., 2011; French et al., 2001). Several (Fraser et al., 2011; Fulkerson et al., 2011; Gillis & Bar-Or, 2003; Kelishadi et al., 2003) but not all (French et al., 2001; Nicklas et al., 2004) cross-sectional studies on this topic have shown that away-from-home food and fast food intake is inversely related to overweight/obesity in children and adolescents. A recent study on the association between the purchasing frequency of away-from-home food sources for family dinners and weight status and %BF among adolescents and their parents showed that the odds of overweight/obesity were considerably greater when families reported at least one away-from-home dinner purchase in the past week (odds ratio = 1.2 to 2.6) (Fulkerson et al., 2011). Moreover, in the same study, mean %BF was significantly greater for families with weekly purchases of family dinners from fast-food restaurants and takeout sources.

Only three longitudinal studies have been identified on the relationship between away-from-home food and fast food consumption and obesity in children and adolescents (Niemeier et al., 2006; Taveras, Berkey, et al., 2005; Thompson et al., 2004). Taveras et al. (2005) observed, during a period of one year, a significant increase in BMI among children aged 9 to 14 who increased consumption of fried foods away-from-home from less than once per week to 4-7 times per week. Similarly, results from another prospective study among girls aged 8 to 12 at baseline and 11 to 19 years old at follow-up showed a larger mean increase in BMI Z-score in girls who ate fast food twice per week or more at baseline, compared to those who ate fast food once per week or not at all (Thompson et al., 2004). Data from the National Longitudinal Study of Adolescent Health reported that more numerous days of fast food consumption between the ages of 11 and 21 predicted an increase in BMI Z-score between the ages of 18 and 27 (Niemeier et al., 2006). Thus, the latter findings suggest that fast food consumption during adolescence is associated with increased weight gain during the transition to adulthood.

Dairy products

Dairy products are key contributors to dietary quality, since, in a mixed diet, they provide considerable amounts of nutrients, such as magnesium, vitamin B12, zinc, riboflavin and calcium. Significantly, they provide more than half of total calcium intake (Araujo et al., 2011; Miller et al., 2001).

A large survey reported that milk consumption among US adolescents declined 36% from 1965 to 1996, which was not compensated for by an increased consumption of other dairy products (Cavadini et al., 2000). On the other hand, in the DONALD study, reduced consumption of 'fluid milk' (i.e., plain milk, chocolate milk, yogurt drinks, buttermilk) was compensated for by an increased consumption of ‘yogurt’ (i.e., milk for food preparation, yogurt, kefir, soft cheese, custard and other milk desserts) (Alexy & Kersting, 2003). Cross-sectional studies have also examined patterns of dairy intake in children and adolescents (Diethelm et al., 2012; Gharib & Rasheed, 2011). According to the recent results of a Healthy Lifestyle in Europe by Nutrition in Adolescence (HELENA) study, adolescents eat less than two-thirds of the recommended amount of milk (and dairy products), compared with Optimized Mixed Diet and Food Guide Pyramid guidelines (Diethelm et al., 2012). Gharib et al. (2011) showed that daily consumption of milk and its products decreased with age; 65.1% of children aged 7 to 10, 54.7% of adolescents aged 11 to 15 and 37.6% of adolescents aged 15 to 18 ate dairy products daily. These results suggest that a great majority of adolescents do not meet the minimum recommendations for dairy intake.

The concomitant increase in childhood obesity and decrease in dairy consumption, particularly milk, has led researchers to hypothesize that the two may be etiologically related (Huang & McCrory, 2005). Indeed, growing evidence suggests that dairy consumption has a protective effect against obesity. However, the results are very inconsistent across studies, and few have examined the effects of dairy intake on obesity and body composition in adolescents. In the subsequent paragraphs we will describe available studies in children and adolescents accordingly to their design.

Table 2 shows the characteristics of the 19 cross-sectional studies that examined the association between dairy consumption and overweight/obesity in children and adolescents. Most studies explored this association in children 14 years old or younger (Barba et al., 2005; Bessa et al., 2008; Black et al., 2002; Fiorito et al., 2006; Hirschler et al., 2009; Keller et al., 2009; LaRowe et al., 2007; O'Connor et al., 2006; Olivares et al., 2004; Rockett et al., 2001;

Roseman et al., 2007; Tanasescu et al., 2000; Wiley, 2010) and reported BMI as their outcome measure, while few reported body fat (Fiorito et al., 2006), sum of skinfolds (Bradlee et al., 2010; Moore et al., 2008) and/or WC (Bradlee et al., 2010; Hirschler et al., 2009; Keller et al., 2009) as their outcome. Thirteen of the 19 studies reported a protective association (Barba et al., 2005; Black et al., 2002; Bradlee et al., 2010; Fiorito et al., 2006; Forshee & Storey, 2003; Hirschler et al., 2009; LaRowe et al., 2007; Moore et al., 2008; Murphy et al., 2008; Olivares et al., 2004; Rockett et al., 2001; Roseman et al., 2007; Tanasescu et al., 2000), while 4 reported no significant association (Bessa et al., 2008; Keller et al., 2009; Nogueira & Sichieri, 2009; O'Connor et al., 2006) and 2 reported increased risk of overweight/obesity as the result of dairy consumption (Matthews et al., 2011; Wiley, 2010). Moore et al. (2008) explored data from the third and 1999-2002 National Health and Nutrition Examination Surveys (NHANES) and found, among adolescents aged 12 to 16, that low dairy intake (< 1 serving/day for girls and < 2 servings/day for boys) was associated with higher BMI and subcutaneous fat. In addition, Bradlee et al. (2010) reanalyzed the data from NHANES III and showed that mean dairy intake was inversely related to central obesity only in adolescents. However, in both of the studies mentioned above, no consistent association was seen between dairy consumption and body composition among younger children (aged 5 to 11).

Table 2 - Characteristics and outcome measures of cross-sectional studies examining the association between dairy consumption and obesity in children and adolescents.

References	Study details	Dietary assessement	Identification of dairy	Outcomes measures	Main findings
Tanasescu et al. (2000)	n=53 prepubertal children Aged 7 to 10	FFQ	Dairy products include milk, yogurt and cheese	BMI	Multivariate logistic regression analyses indicated that lower dairy product intake (OR = 0.41, 95% CI: 0.19–0.93) was associated with obesity.
Rockett et al. (2001)	n=16,787 Aged 9 to 14	FFQ	Dairy products include instant breakfast drink, yogurt, cottage cheese, cheese, milk, chocolate milk, pudding, frozen yogurt, ice cream, milk shake, cheeseburger, grilled cheese, macaroni and cheese, pizza	BMI	Overweight girls consumed fewer serving per day of dairy products than the non-overweight girls (2.92 vs. 3.05, respectively, $P = 0.04$).
Black et al. (2002)	n=250 (50 milk avoiders and 200 milk-drinking control children) Aged 3 to 10	FFQ	Milk	BMI	The milk avoiders had higher BMIs than the control children ($P<0.01$).
Forshee and Storey (2003)	n=3,311 Aged 6 to 19	24-hour recall	Milk	BMI	BMI has a slight negative association with milk consumption only in girls (b = -4.2, $P<0.05$)
Olivares et al. (2004)	n=1,701 Aged 8 to13	FFQ	Dairy products include milk and yogurt	BMI	Dairy products consumption were significantly greater among the nonobese for both genders across age groups ($P<0.05$).
Barba et al. (2005)	n=884 Aged 3 to 11	FFQ	Milk	BMI	The frequency of consumption of milk was inversely associated ($t = –2.964$, $P = 0.003$) with age- and sex-specific BMI Z-scores by linear regression analysis, controlling for sex, age, PA, birth weight and parental overweight and education.

Table 2 – *continued.*

References	Study details	Dietary assessement	Identification of dairy	Outcomes measures	Main findings
Fiorito et al. (2006)	n=172 girls Aged 11	3 x 24-hour recall	Dairy products include milk, milk-based desserts, yogurt, cheese and calcium-fortified soy milk	BMI Body fat (DXA)	In the total sample, girls who consumed ≥ 3 serving per day of dairy foods had lower %BF and BMI Z-score than girls who ate < 3 serving per day (body fat: 25.9±6.8 vs. 28.3±6.9%; BMI Z-score: 0.3±0.9 vs. 0.6±0.9, respectively, *P*<0.05 for all). Among plausible reporters of energy intake, no relationship between dairy intake and weight status was noted.
O'Connor et al. (2006)	n=1,160 Aged 2 to 5	24-hour recall	Milk and flavored milk	BMI	There was no significant association between the types of milk (percentage of fat and flavored) consumed and BMI.
LaRowe et al. (2007)	n=1,334 Aged 2 to 11	24-hour recall	High-fat milk pattern includes whole or 2% (white or chocolate) fluid milk; whole or 2% evaporated, condensed or buttermilk	BMI	BMI was significantly lower in the high-fat milk pattern compared to the water, sweetened drinks, and soda patterns (adjusted mean BMI = 17.8, 19.9, 18.7, and 18.7, respectively, *P*<0.05), only for children aged 6 to 11.
Roseman et al. (2007)	n=4,049 Aged 11 to14	7-day food recall	Milk	BMI	Overweight students had a significantly lower consumption of milk than all other students (χ^2= 31.67, *P* = 0.002).
Bessa et al. (2008)	n=1,675 Aged 5 to 10	FFQ	Milk	BMI	No significant association was found between milk intake and overweight in both genders.
Moore et al. (2008)	NHANES III: n=3,864 children and 1,884 adolescents NHANES 1999-2002 n=2,231 children and 2,636 adolescents Aged 5 to 11 and 12 to 16	24-hour recall	Dairy products include milk, yogurt and cheese	BMI ∑two skinfolds (triceps and subscapular)	Adolescents in the lowest category of dairy intake had a higher BMI and more subcutaneous fat in the subscapular and triceps skinfolds (*P*<0.05). Among children there was no consistent adverse or protective effect of dairy intake on adiposity.

Table 2 – *continued.*

References	Study details	Dietary assessement	Identification of dairy	Outcomes measures	Main findings
Murphy et al. (2008)	n=7,557 Aged 2 to 18	24-hour recall	Plain and flavored milk	BMI	Only in 12- to 18-year-old boys and girls, mean BMIs and BMI Z-scores among milk drinkers were comparable to or lower than mean measures among nondrinkers of milk (*P*<0.05).
Hirschler et al. (2009)	n=365 Mean age 10.6±2.3 years old	Questionnaire about daily consumption of food groups	Milk	BMI WC	Children who consumed ≥ 4 glasses per day of milk had lower mean value of WC than children who consumed 2-3 glasses and ≤ 1 glasses per day (*P* = 0.026). There were no significant differences in the mean values of BMI among the groups.
Keller et al. (2009)	n=126 twins Aged 3 to 7	Video of two *ad libitum* lunches	Milk includes whole white milk and regular-fat chocolate milk Dietary calcium	BMI WC	Milk or calcium intake was not inversely associated with BMI Z-score (*P* values ranging from 0.30 to 0.80) or with WC (*P* values ranging from 0.13 to 0.53).
Nogueira and Sichieri (2009)	n=1,423 Aged 9 to 16	FFQ 24-hour recall	Milk includes whole, reduced fat and nonfat milk	BMI	There were no significant associations between BMI and frequency of milk intake in both genders.
Bradlee et al. (2010)	n=3,761 children and 1,803 adolescents Aged 5 to 11 and 12 to 16	24-hour recall	Dairy products include milk, yogurt and cheese	WC ∑ subscapular and suprailiac skinfold thicknesses	In adolescent (12-16 years old) boys and girls, WC and the sum of subscapular and suprailiac skinfold thicknesses were inversely associated with mean dairy intake (*P*<0.05).
Wiley (2010)	n=1,493 younger children and 2,526 children Aged 2 to 4 and 5 to 10	24-hour recall	Milk includes plain and flavored milk, buttermilk, reconstituted powdered milk Dairy products include milk, yogurt, cheese and ice-cream	BMI	Younger children (2-4 years old) in the highest quartile of dairy and milk intake had higher BMIs than those in the lowest quartiles. Among children of 5–10 years of age dairy intake had no relationship to BMI and those in highest quartile for milk intake had higher BMIs than those in quartile two.
Matthews et al. (2011)	n=1,764 Aged 6 to 19	FFQ	Dairy products include whole and chocolate milk, cottage cheese, cheeses, yogurt, pudding, ice cream, frozen yogurt, milk shake	BMI	Subjects who were in the highest quartile of dairy consumption had significantly higher odds of overweight than those in the lowest quartile (OR = 1.99, 95% CI: 1.34- 2.94).

BMI, body mass index; %BF, percent body fat; CI, confidence interval; DXA, dual energy x-ray absorptiometry; FFQ, food frequency questionnaire; NHANES, National Health and Nutrition Examination Surveys; OR, odds ratio; PA, physical activity; WC, waist circumference.

Prospective studies that have investigated the relationship between dairy intake and change in body composition are presented in Table 3. Carruth and Skinner (2001) evaluated the effects of preschool food consumption, including dairy foods, on body composition and reported an inverse association between daily servings of dairy consumption and body fat. Likewise, Johnson et al. (2007) found that each serving of milk between the ages of 5 and 7 years was associated with a decrease in body fat mass at age 9. The results of the Framingham Children's Study also showed that lower consumption of dairy products during early childhood was associated with increased risk of gaining excessive amounts of body fat by early adolescence (Moore et al., 2006). However, there are other prospective studies in which no associations have been found (Fiorito et al., 2009; Huh et al., 2010; Newby et al., 2004; Phillips et al., 2003; Striegel-Moore et al., 2006; Tam et al., 2006). For instance, Phillips et al. (2003) reported that there was no relationship between BMI Z-score or %BF and dairy consumption over time. Newby et al. (2004) also found that annual change in weight and BMI were not significantly related to intake of milk. On the other hand, one study found that children who reported higher total milk intake experienced larger BMI gains, although this appeared to be mediated by energy intake (Berkey et al., 2005).

Table 3 – Characteristics and outcome measures of prospective studies examining the association between dairy consumption and obesity in children and adolescents.

References	Study details	Dietary assessement	Identification of dairy	Outcomes measures	Main findings
Carruth and Skinner (2001)	n=53 Aged at baseline 24 months old Duration of follow-up=3 years and 10 months	3-day food record for each of six interviews	Type of dairy product not specified Dietary calcium	Body fat (DXA)	General linear models showed that mean longitudinal calcium intake and total servings of dairy products were associated negatively with %BF or grams of total body fat (P<0.0001).
Phillips et al. (2003)	n=178 nonobese premenarcheal girls Aged at baseline 8 to 12 Duration of follow-up=until 4 years postmenarche	FFQ at each annual follow-up visit	Dairy foods include skim/low-fat and whole milk, cream, sherbet or ice milk, ice cream and sundaes, milkshakes, yogurt, cottage, ricotta, cream cheese, and other cheeses Dietary calcium from dairy food	BMI Body fat (BIA)	Linear mixed effects modeling indicated no relationship between BMI Z-score or %BF and measures of dairy food or calcium consumption.
Newby et al. (2004)	n=1,345 Aged at baseline 2 to 5 Duration of follow-up=12 months	FFQ at 6 and 12 months of follow-up	Milk	Body weight BMI	In multivariate regression analyses adjusted for age, sex, energy intake, change in height, and additional sociodemographic variables, weight change and BMI were not significantly related to intake of milk.
Berkey et al. (2005)	n=12,829 Aged at baseline 9 to 14 Duration of follow-up=3 years	FFQ at each year	Milk (white and chocolate) Dairy fat was calculated from milk, butter and cheese	BMI	Children who drank more than 3 servings per day of milk gained more in BMI than those who drank smaller amounts. Multivariate analyses suggested that energy was the most important predictor of weight gain.
Rockell et al. (2005)	n=46 Aged at baseline 3 to 10 Duration of follow-up=2 years	FFQ 4-day food record	Milk	BMI	Children milk avoiders had higher BMI values than the reference population (milk drinkers).

Table 3 – *continued.*

References	Study details	Dietary assessement	Identification of dairy	Outcomes measures	Main findings
Moore et al. (2006)	n=92 Aged at baseline 3 to 6 Duration of follow-up=8 years	15 days of diet records collected before age 6 3-day food record in each subsequent year	Dairy products include milk, yogurt and cheese	BMI Body fat (triceps, subscapular, suprailiac and abdominal skinfold thicknesses)	Children in the lowest sex-specific tertile of dairy intake during preschool (i.e., < 1.25 servings per day for girls and < 1.70 servings per day for boys) had significantly greater gains in body fat during childhood. By the time of early adolescence, those in the lowest tertile of dairy intake had a BMI that was approximately two units higher and an extra 25 mm of subcutaneous fat.
Striegel-Moore et al. (2006)	n=2,371 girls Aged at baseline 9 to 10 Duration of follow-up=10 years	3-day food record at each year	Milk includes all kinds of cow's milk and flavored varieties	BMI	An increase of 100 g of dairy intake per day was associated with a 0.002 (SE = 0.006) point decrease in BMI, although not statistically significant.
Tam et al. (2006)	n=281 Mean age at baseline=7.7±0.6 years old Duration of follow-up = 4.0-6.6 years	3-day food record	Milk	BMI	No association was found between milk consumption and weight change (*P* = 0.995)
Gunther et al. (2007)	n=203 Aged at baseline 6 month old Duration of follow-up=7 years	3-day weighed food record	Dairy proteins include protein from cow milk, custard and other milk desserts, yogurt, buttermilk, and cheese	BMI Body fat (biceps, triceps, subscapular, and suprailiac skinfold thicknesses)	Dairy protein intake (% of energy) at 12 months of age was positively related to %BF and BMI SD score at the age of 7.
Johnson et al. (2007)	n=521 aged 5; 682 aged 7 Aged at baseline 5 and 7 Duration of follow-up=2 to 4 years	3-day food record	Milk includes all milk consumed as a drink throughout the day and milk added to cereal, tea, coffee, hot chocolate, and milkshakes	Body fat (DXA)	Each serving of milk at the age of 5 and 7 was associated with a -0.51 (95% CI: -0.86 to -0.16, *P*<0.01) and -0.35 (95% CI: -0.51 to -0.14, *P*<0.01) change in body fat mass at the age of 9, respectively.

Table 3 – *continued.*

References	Study details	Dietary assessement	Identification of dairy	Outcomes measures	Main findings
Fiorito et al. (2009)	n=166 girls Aged at baseline 5 Duration of follow-up=10 years	3 x 24-hour recall at each assessment	Milk (plain and flavored)	BMI Body fat (DXA and triceps and subscapular skinfold thickness) WC	Milk intake at age 5, was not a predictor of body fat, BMI or WC from age 5 to 15.
Huh et al. (2010)	n=852 Aged at baseline 2 Duration of follow-up=1 year	FFQ	Milk includes whole, reduced fat (2%), 1%, nonfat milk, breast milk, formula, soy, others Dairy products include milk, cheese, cream and cottage cheese, yogurt, ice cream, and pudding.	BMI	Neither total milk nor total dairy intake at the age of 2 was associated with BMI Z-score or incident overweight at the age of 3.

BIA, bioelectrical impedance analysis; BMI, body mass index; %BF, percent body fat; DXA, dual energy x-ray absorptiometry; FFQ, food frequency questionnaire; SD, standard deviation; SE, standard error; WC, waist circumference.

Few experimental studies have examined the relationship between dairy consumption and fatness or body weight in children and adolescents. The majority of these studies were originally designed to assess the effects of dairy intake on bone health, with body composition being a secondary outcome. Table 4 presents the experimental studies that examined the effects of introducing dairy products into children's and/or adolescents' diets on body composition and weight changes. Most studies did not find any association between dairy intake and body composition and/or weight (Cadogan et al., 1997; Chan et al., 1995; Ghayour-Mobarhan et al., 2009; Lau et al., 2004; Merrilees et al., 2000; St-Onge et al., 2009; Volek et al., 2003). Kelishadi et al. (2009) conducted a trial of 95 obese prepubescent children who, in addition to attending 6 consecutive monthly family-centered education sessions about healthy lifestyles, were randomly assigned to 3 groups: an isocaloric dairy-rich diet (> 800 mg calcium per day), a calorie-restricted diet (-500 kcal per day) or a control diet group (i.e. without additional recommendations). Follow-ups were then conducted with each of these groups twice a year for 3 years. In all groups, BMI standard deviation score and WC decreased significantly after the 6-month trial. Although there was a rise in BMI and WC during the follow-up period and until the end of the study in all groups, the rise was significantly lower in the dairy-rich diet group, as compared to the two other groups.

Table 4 - Characteristics and outcome measures of experimental studies examining the association between dairy consumption and obesity in children and adolescents.

References	Study details	Intervention details	Outcomes measures	Main findings
Chan et al. (1995)	n=48 girls Aged at baseline 9 to 13 Duration of study=12 months	One group's diet was supplemented with dairy products for the recommended dietary allowance of 1200 mg calcium daily. The control group ate their usual diet.	Body fat (DXA) Lean body mass (DXA)	There were no differences between the two groups in lean mass and body fat.
Cadogan et al. (1997)	n=80 girls Mean age at baseline=12.2±0.3 years old Duration of study=18 months	The intervention comprised 568 ml of whole or reduced fat milk as a daily supplement to their usual food intake. Subjects in the control group were asked to continue with their habitual diet.	Body weight Body fat (DXA) Lean body mass (DXA)	No significant differences were found between the two groups regarding body weight, %BF, body fat mass and lean body mass.
Merrilees et al. (2000)	n=91 girls Aged at baseline 15 to 16 Duration of study=3 years (2 years of supplementation + 1 year follow-up after the end of supplementation)	The girls were randomly allocated to either the control group or the supplemented group. The supplemented group was supplemented with dairy products for at least 1000 mg per day and the dairy supplements were delivered fortnightly. Dairy products included milk, flavored milk, dairy dessert, cheese or yogurt; low fat options were available.	Body weight Body fat (DXA) Lean body mass (DXA)	No differences were seen in the changes between the two groups from baseline, the end of supplementation or 1 year follow-up for weight, body fat mass and lean body mass.
Volek et al. (2003)	n=28 boys Aged at baseline 13 to 17 Duration of study=12 weeks	In addition to their habitual diet, adolescents consumed 3 servings per day of 1% fluid milk (n=14) or juice not fortified with calcium (n=14) while engaged in a 12-week resistance-training program.	Body weight Body fat (DXA) Lean body mass (DXA)	There were no differences between the two groups in body weight, %BF, body fat mass and lean mass.
Du et al. (2004)	n=681 girls Aged at baseline 10 Duration of study=2 years	Schools were randomized into three groups: group 1, 207 girls consumed a carton of 330 ml milk fortified with calcium on school days over the study period; group 2, 240 girls received the same quantity of milk additionally fortified with 5 or 8 µg cholecalciferol; group 3, 234 control girls.	Body weight Body height	The percentage increase in height, sitting height and body weight after 2 years in the girls in the two supplemented groups were significantly greater compared to the girls in the control group (*P*<0.01).

Table 4 – *continued.*

References	Study details	Intervention details	Outcomes measures	Main findings
Lau et al. (2004)	n=324 Aged at baseline 9 to 10 Duration of study=18 months	Participants were randomized in one of three groups: 40 g high-calcium milk powder (650 mg of calcium), 80 g high-calcium milk powder (1300 mg of calcium), or control. The milk supplement was given daily to the treatment groups.	Body weight Body fat (DXA) Lean body mass (DXA)	There were no significant differences when comparing the 80 g or 40 g milk powder supplementation group with control group in terms of body weight, body fat mass and lean body mass changes.
Albala et al. (2008)	n=93 overweight/obese children Aged at baseline 8 to 10 Duration of study=16 weeks	Participants were randomly assigned in intervention group and control group. Intervention consisted in replacing SSB's consumption to 3 servings per day of milk (1 serving ≈ 200 g) delivered to their homes.	Body weight Body height Body fat (DXA) Lean body mass (DXA)	Change in %BF did not differ significantly between the groups ($P = 0.22$). Lean mass accretion was greater in the intervention group than in the control group ($P = 0.04$). For boys, but not for girls, height increased more in the intervention group than in the control group ($P<0.01$).
Ghayour-Mobarhan et al. (2009)	n=96 overweight/obese children Aged at baseline 12 to 18 Duration of study=12 weeks	Participants were randomized to receive a calorie restricted diet providing a 500 kcal per day deficit from total energy expenditure and two (n=35), three (n=28) or four (n=33) servings of dairy products per day.	Body weight BMI Body fat (BIA)	Significant reductions in overall BMI, BMI Z-score, weight, total %BF and total body fat mass were observed ($P<0.001$), but they were not significantly affected by increasing dairy intake ($P>0.05$).
Kelishadi et al. (2009)	n=95 obese prepubescent children Mean age at baseline=5.6±0.5 years old Duration of study=3 years	In addition to attending 6 consecutive monthly family-centered education sessions about healthy lifestyle, an isocaloric dairy-rich diet (> 800 mg calcium per day) was recommended to the children of one group (DR: dairy-rich diet), the second group was placed on a calorie-restricted diet (ER: energy-restricted), and the third group received no additional recommendation (C: controls).	BMI WC Body fat	In all groups, BMI SD score and WC decreased significantly after the 6-month trial, but had a sustained significant rise during the follow-up period until the end of the study; however, in the DR group, this rise was significantly lower than in the other groups. After the trial, %BF decreased significantly in all groups but there were no differences between groups.
St-Onge et al. (2009)	n=45 overweight children Aged at baseline 8 to 10 Duration of study=16 weeks	Participants were randomized to either high (4 x 236 ml per day) or low (1 x 236 ml per day) milk consumption. Children were provided dietary counseling on healthy eating at baseline and at week 1, 2, 4, 6, 8, and 12.	Body weight Intermuscular, subcutaneous, visceral and total adipose tissue (MRI)	Body weight changes during the16-week study did not differ between the high-milk (1.3±0.3 kg) and low-milk (1.1±0.3 kg) groups. There was no beverage x week interaction on any of the body composition.

BIA, bioelectrical impedance analysis; BMI, body mass index; %BF, percent body fat; DXA, dual energy x-ray absorptiometry; MRI, magnetic resonance imaging; SD, standard deviation; SSB, sugar-sweetened beverage; WC, waist circumference.

Several studies have suggested the beneficial effects of some dairy components, especially calcium, on body weight and body fat loss. The plausible mechanism most frequently cited relates to the effects of calcium on adipocyte metabolism and fatty acid absorption from the gastrointestinal tract (Dougkas et al., 2011). Zemel (2003, 2004) has demonstrated that the concentration of intracellular Ca^{2+} in human adipocyte is increased by the stimulation of parathyroid hormone and 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$), which occurs in response to a low-calcium diet. The resultant increase in intracellular Ca^{2+} exerts a coordinated effect on adipocyte lipid metabolism, stimulating lipogenic gene expression and lipogenesis, thereby increasing lipid filling and adiposity. In addition, decreased $1,25(OH)_2D_3$ may increase the expression of uncoupling protein-2 (UCP2) via nuclear vitamin D receptor in white adipose tissue and thus might contribute to improved thermogenesis (Dougkas et al., 2011; Shi et al., 2002; Zemel, 2004). However, the role of UCP2 in thermogenesis is not clear, and unknown mechanisms may lead to this effect. On the other hand, the regulation of both UCP2 and intracellular Ca^{2+} by $1,25(OH)_2D_3$ appears to also modulate adiposity by affecting apoptosis via a dose-dependent mechanism (Sun & Zemel, 2004a, 2004b). Another explanation for the beneficial effects of calcium on body composition is its role in glucocorticosteroid metabolism. Local adipose tissue glucocorticoid levels and intracellular glucocorticoid availability are controlled by the activity of 11-β-hidroxysteroid dehydrogenase type 1 (11-β-HSD-1), which can generate active cortisol from inactive cortisone (Zemel & Sun, 2008). The decrease in $1,25(OH)_2D_3$ down-regulates 11-β-HSD-1 expression and cortisol release, which consequently decreases the size of adipose fat deposits (Morris & Zemel, 2005). An additional role of $1,25(OH)_2D_3$ includes effects on adipocyte differentiation and proliferation via the regulation of reactive oxygen species and inflammatory cytokines (Sun et al., 2008). Calcium is also able to increase the faecal excretion of fat via the formation of insoluble fatty acid soaps or by binding of bile acids (Christensen et al., 2009; Dougkas et al., 2011). On the other hand, evidence suggests that calcium may mediate the increased fat oxidation, but results remain controversial (Melanson et al., 2005).

Yet, these mechanisms do not entirely explain the observed "antiobesity" effects of dairy products (Zemel, 2005). It has been noted that calcium from dairy products has greater effects on body weight regulation than supplemental calcium, which would lead to the hypothesis that dairy foods can influence body adiposity by calcium-independent mechanisms (Dougkas et al., 2011; Zemel et al., 2004).

Dairy products are an important source of proteins: caseins (α-, β-, κ-, γ-casein) and whey proteins (β-lactoglobulin, α-lactalbumin, serum albumin, immunoglobulins, lactoferrin, lactoperoxidase) which constitute approximately 80% and 20% of such products, respectively (Shah, 2000). Whey proteins have been positively associated with satiety, stimulating the release of anorexic peptides (Dougkas et al., 2011). Moreover, it has been shown that whey proteins inhibit angiotensin-converting enzyme and consequently inhibit the production of angiotensin II hormone (Zemel, 2005), which has been reported to up-regulate adipocyte lipogenesis, resulting in the inhibition of fat deposition (Huth et al., 2006).

The carbohydrates contained in dairy products, particularly in milk, may also contribute to satiety and appetite regulation. Lactose is the principal carbohydrate in milk (Miller et al., 2007) and is traditionally classified as a low-glycaemic index carbohydrate. As seen previously, it has been suggested that low-glycaemic index foods may increase satiety and reduce energy intake by affecting blood glucose concentration and by stimulating gut peptides such as CCK, glucagon-like peptide-1 (GLP-1) and peptide tyrosine tyrosine (PYY) (Dougkas et al., 2011; Kong et al., 2011; Roberts, 2000).

Dairy products are also a representative food source of CLA. Although the impact of CLA on body fat is controversial, studies suggest that CLA can inhibit fatty acid synthase, enhance fat oxidation and thermogenesis, and reduce lipogenesis and preadipocyte differentiation and proliferation (Li et al., 2008; Wang & Jones, 2004).

Median chain fatty acids, such as capronic, caprylic, capric and lauric acids, are naturally present in milk (Jensen, 2002). Increased intake of the median chain fatty acids has been associated with decreased lipogenesis and triacylglyceride synthesis (Moussavi et al., 2008) and down-regulation of adipogenic genes and peroxisome proliferator-activated receptor-gamma (PPAR-γ) (Cock et al., 2004).

The mechanisms underlying the effects of dairy product intake on body adiposity are summarized in Figure 1.

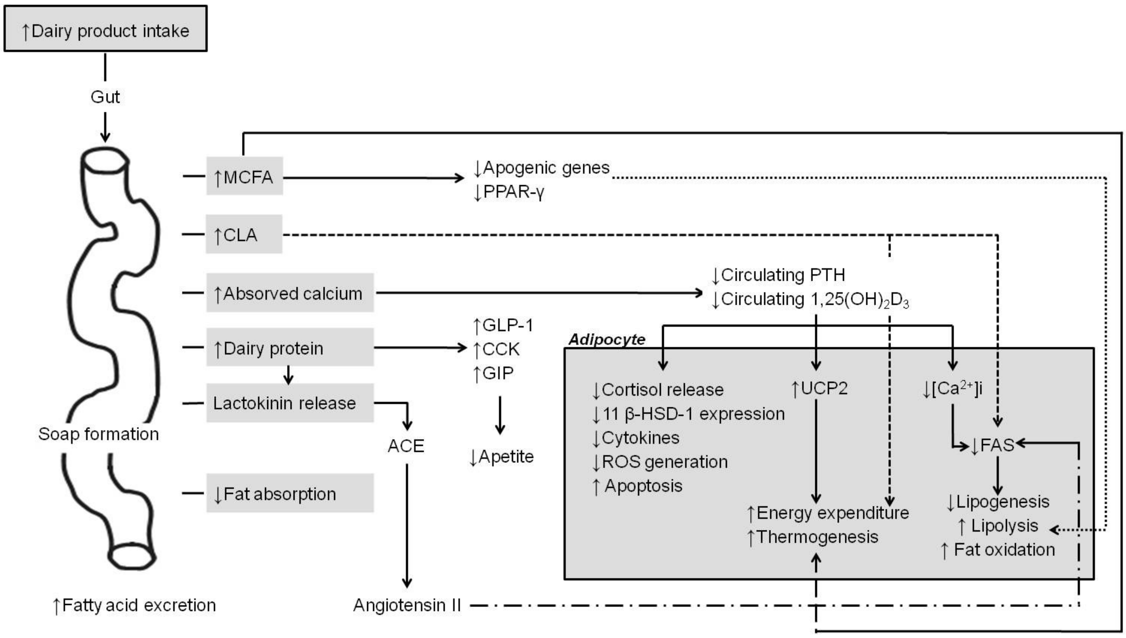

Figure 1 – Mechanisms underlying the effects of dairy product intake on adiposity (adapted from Dougkas et al (2011) and Scholz-Ahrens & Schrezenmeir (2006)). ↑, increased; ↓, decreased; ACE, angiotensin-converting enzyme; $[Ca^{2+}]_i$, intacellular calcium; CCK, cholecystokinin; CLA, conjugated linoleic acid; FAS, fatty acid synthase; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide-1; MCFA, medium chain fatty acids; PPAR-γ, peroxisome proliferator-activated receptor-gamma; PTH, parathyroid hormone; ROS, reactive oxygen species; UCP2, uncoupling protein-2; $1,25(OH)_2D_3$, 1,25-dihydroxyvitamin D_3; 11-*β*-HSD-1, 11-*β*-hidroxysteroid dehydrogenase type 1.

It is worth mentioning that no study has specifically examined these mechanisms in children and adolescents, and the dynamic metabolic changes that occur during growth and puberty may further complicate these issues (Huang & McCrory, 2005).

Dietary patterns

The study of dietary patterns has gained popularity in nutritional epidemiology in recent years. Dietary pattern reflects a combination of many dietary items that together provide a picture of ‘total’ diet (Newby, 2007). Statistical techniques, such as cluster or factor analysis, are used to derive patterns empirically. Alternatively, indices that rank multiple nutritional elements and provide a total score of overall diet quality may also be used to define dietary patterns (Newby, 2007). For example, the Mediterranean diet, traditionally consumed by populations from southern Europe, is characterized by high intake of legumes, fruits, nuts, cereals and olive oil (as the main source of additional fat), moderate intake of fish, seafood, poultry, dairy products (yogurt, cheese), eggs and wine, and low intake of red meat. The association between adherence to the Mediterranean diet and obesity has been explored more in adult populations than with children and adolescents. Schröder et al. (2010) studied a representative national sample of Spanish youth and found that high adherence to the Mediterranean diet was inversely associated with WC and WHtR. Another cross-sectional study reported that a lifestyle pattern characterized by higher adherence to a Mediterranean-style diet, higher eating frequency and breakfast consumption was negatively associated with BMI in a representative sample of Greek children and adolescents aged 3 to 18 (Kontogianni et al., 2010). In a study by Farajian et al. (2011), although no association was seen between the Mediterranean diet and overweight/obesity, children with higher adherence to the Mediterranean diet reported following a healthier diet and having higher physical activity levels. Although the epidemiological evidence is inconsistent, the Mediterranean diet is not related to any increased risk of overweight and obesity, and this dietary pattern may play a role in preventing overweight and obesity (Buckland et al., 2008). It has been proposed that the protective role of the Mediterranean diet may be explained by the increased satiety and satiation that this pattern induces, due to its fiber content, prolonged mastication, increased gastric detention, and enhanced release of CCK, as well as its low glycemic load and low energy density (Schroder, 2007).

Eating behaviors

In addition to types of foods and beverages, specific eating behaviors – breakfast consumption, eating frequency, portion size and family meals - have been associated with the development of obesity.

A growing body of literature suggests that adolescents who regularly eat breakfast are at lower risk of being overweight or obese (Albertson et al., 2007; Deshmukh-Taskar et al., 2010; Szajewska & Ruszczynski, 2010; Timlin et al., 2008). Moreover, breakfast consumption in adolescents is also associated with higher intake of several vitamins and minerals, dietary fiber, fruits, vegetables and dairy products, as well as lower intake of total fat, cholesterol and low-nutrient energy-dense foods (Deshmukh-Taskar et al., 2010; Matthys et al., 2007; Preziosi et al., 1999; Timlin et al., 2008). Breakfast consumption is also related to improved cognitive function and academic performance (Cooper et al., 2011). Yet, according to Newby (2007), it is important to consider that children and adolescents with higher BMI may skip breakfast when trying to lose weight, and skipping breakfast may not be the cause of overweight or obesity. Therefore, experimental studies are clearly needed to clarify the association between breakfast skipping and obesity in adolescents.

A high meal frequency has been associated for some time now with low obesity prevalence (Fabry et al., 1964). It is classically described that eating more often may increase energy expenditure, suggesting that frequency of eating may be a behavioral factor worth targeting for obesity prevention. However, studies that have investigated possible explanations for this metabolic phenomenon have reported that there is no difference in the amount of energy expended when the same amount of food is consumed in a few large meals or in several little ones (Bellisle et al., 1997). On the other hand, the results from recent cross-sectional (Barba et al., 2006; Toschke et al., 2005) and prospective studies (Franko et al., 2008; Ritchie, 2012; Thompson et al., 2006) with pediatric populations show that eating frequency has a beneficial effect on childhood obesity. However, there is currently not enough evidence to support a specific recommendation for the use of this behavior to help manage body weight (U.S. Department of Agriculture & U.S. Department of Health and Human Services, 2010).

The marketing of 'supersize' portions, mainly in fast-food restaurants, is a common practice in many countries. Data from the Nationwide Food Consumption Survey (1977-1978) and the Continuing Survey of Food Intake by Individuals (1989-1991, 1994-1996, and 1998) showed that portion sizes and energy intake for specific food types have increased markedly, with the greatest increases for food consumed at fast food establishments and in the home. For example, in the United States between 1977 and 1996, energy of salty snacks increased by 93 kcal, while the portion size of soft drinks increased by 49 kcal, of hamburgers by 97 kcal, of french fries by 68 kcal and of Mexican food by 133 kcal (Nielsen & Popkin, 2003). Evidence suggests that people poorly estimate portion sizes and that subsequent energy compensation for large meals is incomplete and, therefore, is likely to lead to overconsumption (World Health Organization, 2003). Hence, large portion sizes are a possible causative factor in excessive weight gain (Nielsen & Popkin, 2003). Studies on the effects of large portions of food on children's energy intake and weight status are scarce. McConahy et al. (2004) and Fisher et al. (2007) showed that portion size influences energy intake in pre-school children. In addition, one cross-sectional study reported that meal portion size was positively associated with BMI percentile in boys aged 6 to 11 and in adolescents aged 12 to 19 (Huang et al., 2004) .

Eating meals with one's family relates to numerous healthy dietary habits in adolescents, including higher intake of dietary fiber, several vitamins and minerals, dairy products, and fruits and vegetables as well as more frequent breakfast consumption (Andaya et al., 2011; Fulkerson et al., 2009; Gillman et al., 2000; Neumark-Sztainer et al., 2003; Videon & Manning, 2003). In addition, it has been reported that the frequency of family meals is inversely related to the consumption of fried foods and SSBs (Gillman et al., 2000; Neumark-

Sztainer et al., 2003). Regular family meals give parents the opportunity to monitor and limit children’s intake of calorically dense and ‘junk’ food and to serve as role models for healthy eating behavior. Likewise, regular family meals may help develop healthy eating habits that persist even when the adolescent is not eating with the family (Sen, 2006). However, the frequency with which adolescents eat meals with their families decreases with age, and it has been suggested that the frequency of family meals has also declined due to changes in our society (Gillman et al., 2000; Nicklas et al., 2004). Regarding obesity, cross-sectional (Fulkerson et al., 2009; Gillman et al., 2000; Taveras, Rifas-Shiman, et al., 2005) and prospective studies (Gable et al., 2007; Sen, 2006) have shown an inverse association between family meal frequency and overweight and obesity, but other prospective studies have found no association (Fulkerson et al., 2008; Taveras, Rifas-Shiman, et al., 2005). Thus, according to the findings, family meals may be relevant to the prevention and treatment of overweight and obesity in adolescence.

Chapter 2

Experimental work

Data for the present thesis were derived from a school-based study – The Azorean Physical Activity and Health Study II, which aimed to evaluate physical activity, physical fitness, overweight/obesity prevalence, dietary intake, health-related quality of life and other factors in 15 to 18 years old adolescents. This study was carried out in 6 of the 9 Azorean Islands (S. Miguel, Terceira, Faial, Pico, S. Jorge and Graciosa), where 95% of the Azorean population lives (Instituto Nacional Estatística, 2003).

All participants in this study were informed of its goals, and the parent or guardian of each participant provided written informed consent for his/her child to participate. The study was approved by the Faculty of Sport, University of Porto and the Portuguese Foundation for Science and Technology Ethics Committee; it was conducted in accordance with the World Medical Association's Helsinki Declaration for Human Studies.

The population was selected by means of proportionate stratified random sampling, taking into account location (island) and number of students, by age and sex, in each school. The estimated number of subjects for the representativeness of adolescent population was 1,422, but in order to prevent the collection of incomplete information, data was collected for 1,515 adolescents.

Measurements took place during autumn 2008. Participants were evaluated during school physical education classes by physical education teachers specially trained for the data collection.

Finally, the sample was weighted in accordance with the distribution of the Azorean population in schools and so as to guarantee the real representativeness of each group (by age and gender).

The basic characteristics of each paper which are integrated in this dissertation are shown in Table 1. The detailed description concerning sample size, variables assessment and statistical procedures are presented in the corresponding paper at the materials and methods section.

Table 1 – Summary of the characteristics of the papers integrated in the dissertation.

	Sample size	Age	Dependent variables	Independent variables
Paper I *Food consumption, physical activity and socioeconomic factors related to body mass index, waist circumference and waist-to-height ratio among adolescents*	1,209	15-18 years	BMI WC WHtR	Individual and food groups (dairy, milk, yogurt, cheese, RTECs, fruits, vegetables, vegetable soup, sweets and pastries, SSBs and fast food) Physical activity Socioeconomic factors (parental education and employment status)
Paper II *Association between dairy product intake and abdominal obesity in Azorean adolescents*	903	15-16 years	WC	Dairy products (milk and yogurt)
Paper III *Milk intake is inversely related to body mass index and body fat in girls*	1,001	15-18 years	BMI Body fat	Total dairy products Milk Yogurt Cheese
Paper IV *Relationship of milk intake and physical activity to abdominal obesity among adolescents*	1,209	15-18 years	WC	Milk Physical activity

BMI, body mass index; RTECs, ready-to-eat cereals; SSBs, sugar-sweetened beverages; WC, waist circumference; WHtR, waist-to-height ratio.

Paper I

[Submitted – *under review*]

Food consumption, physical activity and socioeconomic status related to body mass index, waist circumference and waist-to-height ratio in adolescents

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ABSTRACT

Background: There is some evidence that in developed countries, childhood obesity may be associated with low socioeconomic status (SES), low physical activity levels and poor eating habits.

Objective: To examine the association between adolescent obesity and the intake of food groups, physical activity and SES.

Methods: A cross-sectional study was conducted with 1,209 adolescents (503 boys), aged 15-18, from the Azorean Archipelago, Portugal in 2008. Anthropometric measurements were recorded (weight, height, waist circumference-WC). Cole's cut-points were used to categorize body mass index (BMI) as normal weight, overweight and obese. Abdominal obesity was defined by a WC at or above the 90th percentile (WC≥P90th), as well as a waist-to-height ratio (WHtR) at or above 0.500. Adolescent food intake was measured using a semi-quantitative food frequency questionnaire, and consumption of each food group was categorized as 'equal to or above the median' or 'above the median' of the total sample. Physical activity was assessed via a self-report questionnaire, and participants were divided into 'active' and 'low-active' categories. SES was assessed with reference to parental education and employment status. The association between food consumption, physical activity, SES and BMI, WC and WHtR was evaluated using logistic regression analysis, and the results were adjusted for potential confounders.

Results: The proportion of overweight and obese adolescents was 30.9%; the proportion of adolescents with WC≥P90th was 26.8%, and 34.1% of adolescents had WHtR≥0.500. Adolescents with abdominal obesity had lower intake of milk and ready-to-eat cereals (P<0.05, for all). Adolescents with abdominal obesity had a higher proportion of fathers with low levels of education and of fathers and mothers with low employment status (P<0.05, for all). Adolescents with abdominal obesity were also less engaged in physical activity than their lean counterparts (P<0.05, for all). After adjusting for confounders, higher intake of milk and ready-to-eat cereals was negatively associated with abdominal obesity, whereas low paternal employment status was positively associated with abdominal obesity (*P*<0.05, for all). No significant association was found with BMI.

Conclusion: We found that higher milk and ready-to-eat cereal intake and father's employment status had a protective effect on abdominal obesity.

Keywords: diet; physical activity; socioeconomic status; overweight/obesity; adolescents.

INTRODUCTION

Obesity in children and adolescents is a serious public health problem that is associated with enhanced risk of such chronic diseases as diabetes, hypertension and cardiovascular failure (1). Furthermore, obesity in childhood increases the likelihood of obesity and its associated complications in adulthood (1, 2). Different methods have been used to identify obesity in childhood and adolescence, the most common being the body mass index (BMI), which is significantly associated with relative fatness in this age group (1). On the other hand, the use of other simple measures to evaluate adiposity, such as waist circumference (WC) or waist-to-height ratio (WHtR), has also been suggested (3, 4).

Although obesity is a multifactorial disease, dietary intake and physical activity play an important role in the development of this condition in children and adolescents. Evidence suggests that overweight and obese adolescents often have low physical activity levels and excessive or inadequate consumption of specific food groups (5-7). Although the findings are inconsistent, it has been seen that low-nutrient, energy-dense foods (i.e., fast food, sweets and pastries) and beverages (i.e., soda, juice and soft drinks) are associated with enhanced risk of children and adolescents being overweight or obese (8, 9). On the other hand, some studies have identified food groups whose intake has a beneficial effect on obesity, such as fruits, vegetables, ready-to-eat cereals and dairy products (10-12). However, the results regarding the role of specified food groups in the development of overweight/obesity remain controversial in pediatric populations.

Socioeconomic status (SES) has also been associated with childhood obesity. It has been suggested that in developed countries, low SES is associated with increased obesity prevalence, whereas in developing countries, the opposite seems to be true (13).

In light of the fact that childhood obesity is a public health problem worldwide, there is a critical need to identify related risk factors by evaluating the food group intake, physical activity levels and SES of adolescents, as well as the measures of obesity used with this age group. Thus, the aim of this study was to examine the association between the consumption of certain food groups, physical activity, socioeconomic factors and different measures of obesity in adolescents.

MATERIALS AND METHODS

Sampling

Data for the present cross-sectional study were derived from a school-based study – The Azorean Physical Activity and Health Study II, which aimed to evaluate physical activity, physical fitness, overweight/obesity prevalence, dietary intake, health-related quality of life and other factors in 15 to 18 years old adolescents, in 2008. This study was carried out in 6 of the 9 Azorean Islands (S. Miguel, Terceira, Faial, Pico, S. Jorge and Graciosa), where 95% of the Azorean population lives (14).

All participants in this study were informed of its goals, and the parent or guardian of each participant provided written informed consent for his/her child to participate. The study was approved by the Faculty of Sport, University of Porto and the Portuguese Foundation for Science and Technology Ethics Committee; it was conducted in accordance with the World Medical Association’s Helsinki Declaration for Human Studies.

The population was selected by means of proportionate stratified random sampling, taking into account location (island) and number of students, by age and sex, in each school. The estimated number of subjects for the representativeness of adolescent population was 1,422, but in order to prevent the collection of incomplete information, data was collected for 1,515 adolescents. Some adolescents were not included in our analysis (n=306), because information was missing on their dietary intake (n=286), BMI or WC (n=20). This resulted in the collection of data for a total of 1,209 participants (503 boys). The subjects who were excluded from this study did not significantly differ from those who were included, with regard to age (16.2±1.0y vs. 16.1±1.0y, *P*=0.158), parental education (9.1±4.5y vs. 9.1±4.4y, *P*=0.890) and gender (girls: 61.1% vs. 58.4% and boys: 38.9% vs. 41.6%, *P*=0.388). Finally, the sample was weighted in accordance with the distribution of the Azorean population in schools and so as to guarantee the real representativeness of each group (by age and gender).

Anthropometric measures

Body height and body weight

Body height and body weight were determined using standard anthropometric methods. Height was measured to the nearest mm in bare or stocking feet, with adolescents standing upright against a Holtain portable stadiometer (Crymych, Pembrokeshire, UK). Weight was measured to the nearest 0.10 kg, with participants lightly dressed (in underwear and t-shirts), and with the use of a portable digital beam scale (Tanita Inner Scan BC 532, Tokyo, Japan).

BMI was calculated using the ratio of weight/height^2 (kg/m^2). Subjects were classified as normal weight, overweight or obese, according to age- and sex-specific cut-off points specified by the International Obesity Task Force (15, 16). Underweight subjects (2.6%) were combined with subjects in the normal weight category, due to the fact that the former represented a small proportion of the overall sample.

Waist circumference and waist-to-height ratio

For the present study, WC and WHtR were both used as proxy measures of abdominal obesity. WC measurements were taken midway between the tenth rib and the iliac crest and recorded to 0.1 cm. A non-elastic flexible tape measure was used, with subjects standing erect – arms by sides, feet together and abdomen relaxed – as well as without clothing covering the waist area. Subjects were divided into two categories (<90th and ≥90th percentiles), according to age- and sex-specific cut-off points specified by Sardinha et al. (17). Subjects who were in the 90th percentile or above were considered to have abdominal obesity (18). WHtR was calculated as the ratio between WC (in cm) and height (in cm). A WHtR cut-off point of 0.500 was used to define abdominal obesity in males and females (19-21).

Pubertal stage

To determine pubertal stage (which ranged from 1 to 5), each subject was asked to self-assess his/her stage of development of secondary sex characteristics. Breast development in girls and genital development in boys was evaluated according to criteria outlined by Tanner and Whitehouse (22).

Socio-demographic and lifestyle variables

Participants answered a questionnaire that assessed several socio-demographic and lifestyle variables.

Parental education and employment status

For the present study, mother's and father´s education and employment status were both used as proxy measures of SES. Participants were divided into three categories, reflecting divisions within the Portuguese educational system: mandatory or less (≤ 9 school years), secondary (10 to 12 school years) and college/university (> 12 school years). Employment status was divided into four categories, according to the standard Portuguese method of classifying occupations: (i) high employment status (which included members of the armed forces, representatives of legislative branch officers and agencies, officers, directors and executive officers, and specialists in intellectual and scientific occupations), (ii) medium employment status (which included mid-level technicians and professionals, administrative staff, personal service workers, safety and protective service providers and sellers and qualified workers in agriculture, fishery and forestry), (iii) low employment status (which included qualified workers in industry and construction, artisans, operators of machinery and equipment, and unqualified workers), (iv) no relation with employment (which included pensioners, students and the unemployed) (23, 24).

Dietary intake

Dietary intake was measured via a self-administered semi-quantitative food frequency questionnaire (FFQ), validated for the Portuguese population (25). This semi-quantitative FFQ was designed in accordance with criteria laid out by Willett et al. (26) and adapted to include a variety of typical Portuguese food items. The FFQ was adapted for adolescents by including foods more frequently eaten by this age group (27); the adolescent version covered the previous 12 months and comprised ninety-one food items and beverage categories. For each item, the questionnaire offered nine frequency response options, ranging from 'never' to 'six or more times per day', and information on standard portion size and seasonality. Any foods not listed in the questionnaire could be listed by participants in a free-response section. Energy and nutritional intake were estimated with regard to respondents' ratings of the frequency, portion and seasonality of each item, using the software Food Processor Plus (ESHA Research Inc., Salem, OR, US). This program uses nutritional information from the United States that has been adapted for use with typical Portuguese foods and beverages.

For the present study, we defined eleven food groups: (i) dairy (milk, yogurt and cheese), (ii) milk (whole, semi-skimmed and skimmed), (iii) yogurt, (iv) cheese, (v) ready-to-eat cereals, (vi) fruits (fresh fruits, including tropical fruits), (vii) vegetables (cabbage, spinach, broccoli, lettuce, peppers, tomatoes, cucumbers, onions, carrots, etc.), (viii) vegetable soup, (ix) sweets and pastries (other biscuits, apart from simple ones, croissants, doughnuts, cakes, chocolates, chocolate snacks, quince jam, compote, jelly, honey, sugar, candy), (x) fast food (pizza, hamburgers, mayonnaise, salted snacks), (xi) sugar-sweetened beverages (soda, juice, fruit juice). Then, participants were divided into two categories, according to the amount of each food group consumed: one corresponding to intake lower than or equal to the median amount of the total sample and the other corresponding to intake higher than the median amount of the total sample.

Physical activity

Physical activity was assessed via a self-report questionnaire that evaluated leisure-time physical activities (28). This questionnaire has been shown to have good test-retest reliability among Portuguese adolescents (intraclass correlation coefficient: 0.92–0.96) (29). It consists of five questions with four answer choices (each rated on a four-point scale): (1) Outside school, do you take part in organized sports/physical activities?; (2) Outside school, do you take part in non-organized sports/physical activities?; (3) Outside school hours, how many times a week do you take part in sports or physical activities for at least 20 minutes?; (4) Outside school hours, how many hours a week do you usually take part in physical activities, so much that you get out of breath or sweat?; (5) Do you take part in competitive sports? The maximum number of points possible was 20. A physical activity index was obtained for each respondent by totaling his/her points, which corresponded to activity level rankings that ranged from ‘sedentary’ to ‘vigorous’. Participants whose physical activity indices were greater than 10 points were classified as ‘active’, while those whose physical activity indices were 10 points or less were classified as ‘low-active’ (29, 30).

Statistical analysis

The Kolmogorov–Smirnov test was used to verify the normality of the variables. Independent sample t or Mann-Whitney test was performed to compare continuous variables between groups, while the Chi-square test was used for categorical variables. In this report, descriptive analysis is presented in terms of means and standard deviations, unless otherwise stated.

Unconditional logistic regression models were constructed to verify the relationship between overweight/obesity or abdominal obesity (WC≥P90th or WHtR≥0.500) and food group consumption, SES and physical activity. The final model (Model 2) was adjusted for age (in years), gender, pubertal stage, energy intake (in kcal), dietary fiber (in g/1000 kcal) and all of the variables presented in the unadjusted model (Model 1). Age, energy intake and dietary fiber were entered as continuous variables. Furthermore, we adjusted the final logistical model by underreporting energy intake, which was estimated using the ratio between reported energy intake and predicted basal metabolic rate (EI:BMR) (31-33). The thresholds that defined low-energy reporters (underreporters) were 1.70 and 1.71 for girls and boys between 15 and 17 years old and 1.67 and 1.81 for girls and boys age 18. ‘Low-energy reporter’ (a categorical variable) was included in the final model as a confounding factor.

Odds ratios (ORs) and 95% confidence intervals (CIs) were computed for overweight/obesity and abdominal obesity (WC≥P90th or WHtR≥0.500), according to food group intake, physical activity and SES. A *P*-value of <0.05 was regarded as significant. All analyses were performed using IBM SPSS Statistics v.20 (SPSS, Chicago, Illinois, US).

RESULTS

Descriptive characteristics of the adolescents in the sample according to their BMI, WC and WHtR status are shown in Table 1. Adolescents who are classified as overweight/obese or abdominally obese had higher weight, BMI, WC and WHtR values ($P<0.001$, for all). A significantly higher proportion of girls was seen in the abdominal obesity groups ($P<0.05$, for all). Adolescents with abdominal obesity had a higher proportion of fathers with low levels of education and were more likely to have two parents with low employment status ($P<0.05$, for all). Concerning physical activity, adolescents with abdominal obesity were less active than their lean counterparts ($P<0.05$, for all). No significant differences were seen in age between the groups, regardless of their BMI, WC or WHtR status. Our data showed that 16.7% of adolescents reported being in Tanner Stage 3 or lower, while 59.1% reported being in Stage 4 and 24.2% in Stage 5.

Table 1 - Characteristics of the study sample, according to body mass index, waist circumference and waist-to-height ratio status.

	Total (n=1209)	BMI		$P^{b,c}$	WC		$P^{b,c}$	WHtR		$P^{b,c}$
		NW (n=836)	OW/OB (n=373)		<P90th (n=885)	≥P90th (n=324)		<0.500 (n=797)	≥0.500 (n=412)	
Age (years)[a]	16.0 (2.0)	16.0 (2.0)	16.0 (2.0)	0.050	16.0 (2.0)	16.0 (2.0)	0.407	16.0 (2.0)	16.0 (2.0)	0.102
Height (m)[a]	1.65 (0.12)	1.65 (0.13)	1.64 (0.13)	0.312	1.65 (0.13)	1.65 (0.12)	0.675	1.66 (0.13)	1.62 (0.11)	<0.001
Weight (kg)[a]	60.5 (15.0)	56.7 (10.8)	72.5 (17.0)	<0.001	56.6 (12.1)	73.0 (19.1)	<0.001	57.8 (12.7)	66.7 (19.6)	<0.001
BMI (kg/m^2)[a]	22.1 (4.3)	21.0 (2.5)	26.6 (4.1)	<0.001	21.2 (2.9)	26.6 (5.1)	<0.001	20.9 (2.7)	25.6 (5.0)	<0.001
WC (cm)[a]	78.0 (13.0)	75.0 (10.0)	89.0 (13.0)	<0.001	75.0 (9.0)	90.5(9.9)	<0.001	74.0 (8.5)	89.0 (10.5)	<0.001
WHtR[a]	0.468 (0.090)	0.446 (0.060)	0.539 (0.080)	<0.001	0.448 (0.050)	0.559 (0.060)	<0.001	0.443 (0.050)	0.548 (0.060)	<0.001
Gender (%)										
girls	58.4	57.3	60.9	0.256	55.7	65.7	0.002	51.1	72.6	<0.001
boys	41.6	42.7	39.1		44.3	34.3		48.9	27.4	
Father´s education (%)										
mandatory or less	73.2	72.3	75.2		71.1	78.7		70.3	78.8	
secondary	19.8	20.1	19.1	0.502	20.6	17.7	0.019	20.9	17.6	0.004
college/university	7.0	7.6	5.7		8.3	3.6		8.8	3.6	
Mother's education (%)										
mandatory or less	66.1	66.4	65.3		64.7	69.8		63.2	71.7	
secondary	22.6	22.4	23.0	0.944	23.5	20.4	0.316	23.9	20.1	0.020
college /university	11.3	11.2	11.7		11.8	9.8		12.9	8.2	
Father's employment (%)										
high	9.3	9.9	8.0		10.3	6.7		10.6	6.8	
medium	40.6	40.4	40.9	0.381	41.5	38.0	0.067	42.2	37.4	0.023
low	43.8	44.0	43.1		42.6	47.0		41.6	47.9	
no relation with employment	6.3	5.7	8.0		5.6	8.3		5.6	7.9	
Mother's employment (%)										
high	10.1	9.8	10.7		10.2	9.6		10.8	8.6	
medium	41.7	42.3	40.3	0.682	43.2	37.6	0.086	43.5	38.2	0.049
low	46.6	46.5	46.8		45.4	49.9		44.5	50.6	
no relation with employment	1.6	1.4	2.2		1.2	2.9		1.2	2.6	
PAI[a]	13 (8)	13 (8)	12 (7)	0.254	13 (8)	12 (7)	0.001	13.0 (8)	11.0 (7)	<0.001
PAI (%)										
low-active (≤10 points)	35.3	34.3	37.5	0.297	33.3	40.7	0.018	45.9	57.0	<0.001
active (>10 points)	64.7	65.7	62.5		66.7	59.3		54.1	43.0	

[a] data are median (interquartil range); [b] analysis by Mann-Whitney's test for continuous variables; [c] analysis by χ^2 for categorical variables.
BMI - body mass index; NW – normal weight; OW/OB – overweight and obese; PAI – physical activity index; WC - waist circumference; WHtR - waist-to-height ratio.

The energy intake, dietary characteristics and food group consumption of the adolescents, according to their BMI, WC and WHtR status, are presented in Table 2. Adolescents who were overweight/obese or abdominally obese had lower energy intake and lower intake of ready-to-eat cereals, sweets and pastries, fast food and sugar-sweetened beverages, compared to their lean counterparts (P<0.05, for all). Lower dairy and milk consumption was seen in adolescents with abdominal obesity, compared with those classified as without abdominal obesity (P<0.05, for all). There was no significant difference across groups with regard to carbohydrate, total fat, cheese, fruit and vegetable intake.

Table 2 - Dietary characteristics of the study sample, according to body mass index, waist circumference and waist-to-height ratio status.

	Total (n=1209)	**BMI** NW (n=836)	**BMI** OW/OB (n=373)	*P*	**WC** <P90th (n=885)	**WC** ≥P90th (n=324)	*P*	**WHtR** <0.500 (n=797)	**WHtR** ≥0.500 (n=412)	*P*
Energy intake (kcal/day)[a, b]	2301.2 (1431.1)	2341.9 (1465.5)	2060.0 (1282.8)	<0.001	2377.4 (1435.9)	2052.1 (1424.08)	0.001	2386.5 (1535.8)	2065.3 (1399.1)	<0.001
Protein (% of energy)[c, d]	17.8 (3.8)	17.6 (3.8)	18.1 (3.8)	0.021	17.6 (3.7)	18.1 (4.0)	0.094	17.6 (3.7)	18.0 (3.9)	0.122
Carbohydrate (% of energy)[c, d]	1.6 (1.0)	49.4 (7.8)	49.1 (8.0)	0.535	49.4 (7.8)	49.0 (8.1)	0.493	49.3 (7.8)	49.3 (8.0)	0.888
Total fat (% of energy)[c, d]	49.3 (7.9)	32.4 (5.6)	32.1 (5.9)	0.348	32.3 (5.5)	32.2 (5.9)	0.785	32.4 (5.5)	32.1 (5.9)	0.265
Dietary fiber (g/1000 kcal)[a, b]	32.3 (5.7)	9.3 (4.1)	9.7 (4.3)	0.057	9.3 (4.1)	9.7 (3.6)	0.029	9.2 (4.1)	9.7 (4.3)	0.011
Dairy (g/day)[a, b]	400.0 (480.7)	448.5 (481.7)	393.6 (475.8)	0.177	506.8 (480.4)	370.0 (488.2)	0.014	580.1 (475.6)	369.0 (480.0)	0.001
≤ median (%)[e]		48.3	53.9	0.081	47.5	57.1	0.003	46.0	57.8	<0.001
> median (%)		51.7	46.1		52.5	42.9		54.0	42.2	
Milk (g/day)[a, b]	251.9 (430.0)	267.6 (429.2)	251.9 (430.0)	0.190	286.8 (413.2)	250.8 (430.0)	0.004	322.6 (394.2)	250.8 (430.0)	<0.001
≤ median (%)[e]		49.9	53.1	0.319	48.4	57.7	0.004	46.9	58.5	<0.001
> median (%)		50.1	46.9		51.6	42.3		53.1	41.5	
Yogurt (g/day)[a, b]	53.6 (111.6)	53.6 (116.7)	53.6 (107.2)	0.473	53.6 (116.7)	53.6 (107.2)	0.128	53.6 (116.7)	53.6 (107.2)	0.325
≤ median (%)[e]		61.7	57.4	0.162	62.1	55.6	0.040	61.9	57.5	0.154
> median (%)		38.3	42.6		37.9	44.4		38.1	42.5	
Cheese (g/day)[a, b]	12.9 (22.0)	12.9 (20.0)	12.8 (21.8)	0.308	12.9 (19.8)	12.8 (22.0)	0.521	12.9 (25.7)	12.9 (21.6)	0.134
≤ median (%)[e]		62.8	64.9	0.518	63.3	63.9	0.893	62.5	65.3	0.345
> median (%)		37.2	35.1		36.7	36.1		37.5	34.7	
RTECs (g/day)[a, b]	17.1 (34.3)	31.4 (34.3)	17.1 (34.3)	<0.001	31.4 (34.3)	17.1 (34.3)	<0.001	31.4 (34.3)	17.1 (37.3)	<0.001
≤ median (%)[e]		27.6	36.2	0.004	28.0	36.4	0.006	27.0	36.7	0.001
> median (%)		72.4	63.8		72.0	63.6		73.0	63.3	
Fruits (g/day)[a, b]	210.5 (306.5)	207.0 (293.8)	212.8 (323.9)	0.997	204.2 (286.5)	217.3 (331.4)	0.411	203.9 (283.9)	217.3 (329.1)	0.402
≤ median (%)[e]		50.4	49.1	0.709	51.1	46.9	0.217	51.3	47.3	0.203
> median (%)		49.6	50.9		48.9	53.1		48.7	52.7	
Vegetables (g/day)[a, b]	83.2 (125.9)	81.2 (124.7)	86.4 (131.3)	0.131	81.6 (125.4)	86.4 (124.6)	0.379	79.7 (126.0)	87.5 (124.7)	0.388
≤ median (%)[e]		50.7	48.3	0.455	50.6	48.1	0.475	51.1	47.8	0.302
> median (%)		49.3	51.7		49.4	51.9		48.9	52.2	
Vegetable soup (g/day)[a, b]	126.4 (212.1)	126.4 (212.1)	126.4 (212.1)	0.376	126.4 (212.1)	126.4 (212.1)	0.416	126.4 (212.1)	126.4 (212.1)	0.462
≤ median (%)[e]		69.9	67.8	0.500	70.1	67.0	0.325	69.1	69.4	0.948
> median (%)		30.1	32.2		29.9	33.0		30.9	30.6	
Sweets and pastries (g/day)[a, b]	42.3 (55.1)	46.8 (57.7)	31.4 (43.0)	<0.001	44.7 (57.3)	32.3 (43.2)	<0.001	47.1 (57.3)	31.4 (43.1)	<0.001
≤ median (%)[e]		45.7	61.8	<0.001	47.0	60.9	<0.001	44.6	62.3	<0.001
> median (%)		54.3	38.2		53.0	39.1		55.4	37.7	
Fast food (g/day)[a, b]	39.6 (43.1)	40.8 (49.8)	32.3 (35.0)	<0.001	40.3 (48.0)	32.3 (35.0)	0.012	41.3 (49.0)	32.3 (35.4)	0.001
≤ median (%)[e]		46.5	55.8	0.003	46.9	56.2	0.004	45.4	57.0	<0.001
> median (%)		53.5	44.2		53.1	43.8		54.6	43.0	
SSBs (ml/day)[a, b]	253.3 (379.3)	261.0 (393.7)	163.4 (356.8)	0.004	259.3 (403.6)	181.0 (333.3)	0.010	274.2 (401.4)	180.9 (333.3)	0.002
≤ median (%)[e]		49.3	56.8	0.018	49.7	56.8	0.032	48.3	58.0	0.002
> median (%)		50.7	43.2		50.3	43.2		51.7	42.0	

[a] data are median (interquartil range); [b] analysis by Mann-Whitney's test; [c] data are mean (standard deviation); [d] analysis by Student´s t-test; [e] analysis by χ^2 for categorical variables.

BMI - body mass index; NW – normal weight; OW/OB – overweight and obese; RTECs – ready-to-eat cereals; SSBs - sugar-sweetened beverages; WC - waist circumference; WHtR - waist-to-height ratio.

The ORs of overweight/obese and abdominally obese adolescents (WC≥P90th or WHtR≥0.500), according to consumption of the eleven food groups measured, physical activity and SES, are shown in Table 3. Adolescents with milk intake above the median were less likely to express abdominal obesity (WC≥P90th and WHtR≥0.500) than were those with milk intake equal to or below the median (WC≥P90th, OR=0.654, 95%CI: 0.464-0.922; WHtR≥0.500, OR=0.652, 95%CI: 0.468-0.908), after adjusting for other food groups' consumption and sociodemographic variables. Above-the-median intake of ready-to-eat cereals, sweets and pastries and fast food was a negative predictor of abdominal obesity, which was defined by WHtR≥0.500. Adolescents with yogurt intake above the median had higher odds of abdominal obesity, which was defined by WC≥P90th, than did those with an intake equal to or below the median. Above-the-median intake of fruits was a positive predictor of abdominal obesity for both measures of abdominal obesity. Adolescents whose fathers had low employment status and those with fathers had no relation with employment had higher odds of abdominal obesity, which was defined by WHtR≥0.500, compared to adolescents whose fathers had high employment status (low employment status: OR=2.457, 95%CI: 1.047-5.765; no relation with employment: OR=3.115, 95%CI: 1.140-8.510). No significant association was found with BMI.

Table 3 – Odds ratio for overweight/obesity and abdominal obesity (WC≥P90th or WHtR≥0.500) according to food groups intake, physical activity and socioeconomic status.

	Model 1						**Model 2**					
	BMI		**WC**		**WHtR**		**BMI**		**WC**		**WHtR**	
	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
Father´s employment												
high	**Reference**	0.238[a]	**Reference**	0.009[a]	**Reference**	0.002[a]	**Reference**	0.510[a]	**Reference**	0.056[a]	**Reference**	0.018[a]
medium	1.248 (0.776-0.201)		1.408 (0.828-2.396)		1.379 (0.849-2.241)		0.916 (0.424-1.979)		1.401 (0.567-3.463)		1.905 (0.863-4.204)	
low	1.211 (0.754-1.943)		1.698 (1.004-2.869)		1.788 (1.107-2.887)		0.868 (0.382-1.973)		1.576 (0.612-4.060)		**2.457 (1.047-5.765)**	
no relation with employment	1.750 (0.921-3.324)		2.269 (1.137-4.496)		2.170 (1.139-4.136)		1.380 (0.512-3.722)		2.494 (0.836-7.446)		**3.115 (1.140-8.510)**	
Mother´s employment												
high	**Reference**	0.808[a]	**Reference**	0.065[a]	**Reference**	0.052[a]	**Reference**	0.929[a]	**Reference**	0.502[a]	**Reference**	0.403[a]
medium	0.873 (0.565-1.349)		0.926 (0.581-1.475)		1.100 (0.705-1.715)		0.497 (0.225-1.094)		0.587 (0.236-1.459)		0.732 (0.304-1.763)	
low	0.922 (0.600-1.416)		1.168 (0.740-1.843)		1.428 (0.922-2.211)		0.553 (0.235-1.304)		0.698 (0.264-1.843)		0.864 (0.338-2.210)	
no relation with employment	1.474 (0.548-3.967)		2.550 (0.946-6.877)		2.647 (0.988-7.092)		0.994 (0.239-4.130)		1.316 (0.293-5.920)		1.412 (0.321-6.207)	
Father's education												
college/university	**Reference**	0.263[a]	**Reference**	0.007[a]	**Reference**	0.001[a]	**Reference**	0.308[a]	**Reference**	0.426[a]	**Reference**	0.866[a]
secondary	1.401 (0.777-2.524)		2.548 (1.235-5.258)		2.750 (1.410-5.365)		1.598 (0.619-4.126)		1.623 (0.530-4.964)		1.342 (0.468-3.849)	
mandatory or less	1.286 (0.672-2.461)		1.977 (0.904-4.324)		2.065 (1.002-4.257)		1.954 (0.712-5.367)		1.771 (0.546-5.738)		1.202 (0.397-3.642)	
Mother's education												
college/university	**Reference**	0.738[a]	**Reference**	0.147[a]	**Reference**	0.005[a]	**Reference**	0.817[a]	**Reference**	0.822[a]	**Reference**	0.491[a]
secondary	0.940 (0.608-1.453)		1.307 (0.815-2.096)		1.775 (1.117-2.823)		0.930(0.491-1.762)		1.515 (0.588-3.906)		1.808 (0.723-4.518)	
mandatory or less	0.979 (0.598-1.603)		1.051 (0.614-1.799)		1.319 (0.782-2.223)		1.067 (0.525-2.169)		1.602 (0.592-4.331)		1.823 (0.697-4.772)	
PAI	0.870 (0.675-1.121)	0.286[b]	0.727 (0.560-0.945)	0.017[b]	0.646 (0.505-0.826)	0.001[b]	1.000 (0.704-1.421)	0.999[b]	0.983 (0.686-1.409)	0.927[b]	1.027 (0.719-1.437)	0.926[b]
Total dairy	0.800 (0.627-1.022)	0.074[b]	0.679 (0.525-0.877)	0.003[b]	0.624 (0.491-0.793)	<0.001[b]	1.036 (0.740-1.451)	0.838[b]	0.757 (0.535-1.071)	0.116[b]	0.740 (0.529-1.037)	0.080[b]
Milk	0.880 (0.689-1.123)	0.304[b]	0.686 (0.531-0.887)	0.004[b]	0.627 (0.493-0.798)	<0.001[b]	1.145 (0.823-1.593)	0.423[b]	**0.654 (0.464-0.922)**	0.015[b]	**0.652 (0.468-0.908)**	0.011[b]
Yogurt	1.198 (0.935-1-535)	0.153[b]	1.313 (1.015-1.700)	0.038[b]	1.197 (0.940-1.525)	0.145[b]	1.231 (0.879-1.726)	0.227[b]	**1.525 (1.078-2.157)**	0.017[b]	1.329 (0.949-1-861)	0.098[b]
Cheese	0.914 (0.708-1.178)	0.488[b]	0.974 (0.747-1.269)	0.845[b]	0.885 (0.691-1.135)	0.337[b]	1.163 (0.821-1.648)	0.395[b]	1.284 (0.897-1.839)	0.172[b]	1.116 (0.788-1.581)	0.537[b]
RTECs	0.673 (0.519-0.873)	0.003[b]	0.680 (0.519-0.890)	0.005[b]	0.639 (0.495-0.823)	0.001[b]	0.829 (0.581-1.184)	0.303[b]	0.781 (0.541-1.127)	0.186[b]	**0.693 (0.486-0.988)**	0.042[b]
Fruits	1.053 (0.825-1.344)	0.677[b]	1.181 (0.915-1.524)	0.200[b]	1.173 (0.925-1.488)	0.189[b]	1.455 (0.985-2.149)	0.059[b]	**1.704 (1.135-2.557)**	0.010[b]	**1.744 (1.174-2.591)**	0.006[b]
Vegetables	1.103 (0.864-1.409)	0.429[b]	1.104 (0.856-1.424)	0.446[b]	1.139 (0.898-1.445)	0.284[b]	1.056 (0.729-1.529)	0.772[b]	1.214 (0.829-1.780)	0.319[b]	1.391 (0.958-2.018)	0.083[b]
Vegetable soup	1.099 (0.845-1.429)	0.480[b]	1.154 (0.878-1.515)	0.304[b]	0.987 (0.762-1.277)	0.919[b]	1.053 (0.732-1.516)	0.779[b]	1.104 (0.759-1.608)	0.604[b]	0.839 (0.582-1.211)	0.348[b]
Sweets and pastries	0.521 (0.406-0.670)	<0.001[b]	0.569 (0.438-0.738)	<0.001[b]	0.487 (0.381-0.622)	<0.001[b]	0.713 (0.501-1.016)	0.061[b]	0.699 (0.483-1.012)	0.058[b]	**0.591 (0.413-0.845)**	0.004[b]
Fast-food	0.690 (0.540-0.882)	0.003[b]	0.689 (0.533-0.890)	0.004[b]	0.627 (0.493-0.797)	<0.001[b]	0.889 (0.625-1.263)	0.511[b]	0.767 (0.523-1.104)	0.153[b]	**0.698 (0.491-0.992)**	0.045[b]
SSBs	0.738 (0.577-0.943)	0.015[b]	0.752 (0.582-0.972)	0.030[b]	0.676 (0.532-0.860)	0.001[b]	1.150 (0.812-1.636)	0.431[b]	1.185 (0.827-1.699)	0.355[b]	1.155 (0.816-1.635)	0.416[b]

BMI - body mass index; CI - confidence interval; OR – odds ratio; PAI - physical activity index; RTECs - ready-to-eat cereals; SSBs - sugar-sweetened beverage; WHtR - waist-to-height ratio; WC - waist circumference; .

Model 1 – unadjusted model; each variable enters in model separately - the reference group in all food groups items is ≤median of the total sample; PAI – reference is low activity.

Model 2 – adjusted for age, gender, maturation, total energy intake (kcal), low-energy reporters, dietary fiber (g/1000kcal) plus for the other food groups, physical activity and socioeconomic status (parental education and employment status). For total dairy products, milk, yogurt and cheese do not enter in the model.

[a] *P*-value for trend; [b] *P*-value for heterogeneity.

DISCUSSION

The present study explored the relationship between food group intake, physical activity and SES and obesity, as well as different measures of abdominal obesity (i.e., WC and WHtR). The results suggested that the intake of milk and ready-to-eat cereals are negative predictors of abdominal obesity. Few studies have examined the relationship between specific type of dairy intake and measures of abdominal obesity in children and adolescents. In a cross-sectional study with children aged 10, increased milk consumption was associated with lower WC, whereas no significant association was seen with BMI (34). Data from NHANES III also showed that mean dairy intake was inversely associated with central obesity in adolescents (35). A number of possible explanations have been suggested for the protective effect of milk intake on obesity. Milk is an important source of calcium, which appears to play a significant role in the regulation of energy metabolism by reducing the levels of lipogenesis on adipocyte and increasing both faecal fat excretion and fat oxidation (36). It has also been suggested that high-calcium diets are associated with reductions in the size of adipose fat visceral deposits by means of the down-regulation of cortisol release (37). Furthermore, the proteins of milk, especially whey proteins, have been positively associated to satiety (36). On the other hand, however, in our study, yogurt intake seemed to be positively associated with abdominal obesity when the latter was defined as WC≥P90th. The FFQ was not specifically designed to account for different types of yogurt, and a wide variety of yogurts, particularly those with higher fat and sugar content, may complicate the explanation of these findings. Thus, more studies are needed to further examine this association across different types of yogurt nutritional composition.

Concerning ready-to-eat cereals intake, our results are in accordance with evidence that suggests that ready-to-eat cereals consumption protects against childhood obesity. Kafatos et al. (11) found that adolescents who are daily consumers of ready-to-eat cereals had lower mean BMI, WC and WHtR values, compared to non-consumers and occasional consumers of ready-to-eat cereals.

Other cross-sectional (38-40) and prospective studies (41, 42) have shown similar results. According to the latter studies, the ‘antiobesity’ effect of ready-to-eat cereals may be due to the association of these cereals with higher breakfast consumption and milk and dietary fiber intake, which have been associated with lower risk of obesity.

It has been suggested that SES in childhood may influence health behaviors and, thus, predisposition to obesity (43). In the present study, SES was assessed by measuring parental education and employment. After adjustment, only father´s employment status remained a significant predictor of abdominal obesity, as defined by WHtR≥0.500. As recently proposed in a review, father's employment is a probable early marker of the development of obesity in adulthood (13). In particular, the employment of fathers in ‘low status’, ‘blue collar’, ‘unskilled’ and ‘manual’ jobs is classically associated with increased risk of being obese as an adult (13).

In our sample, although physical activity seemed to not be a negative predictor of obesity, adolescents with abdominal obesity were less active than their lean counterparts. Indeed, evidence suggests that low physical activity levels may play a role in the development of abdominal obesity in youth (44). A cross-sectional study with adolescents showed that WC was inversely associated with structured physical activity (outside school, >140 min/week), independent of time spent on sedentary activities (45). Likewise, Ortega et al. (46) found that children and adolescents with low levels of vigorous physical activity had higher odds of having high WC, when compared with those with high levels of vigorous physical activity.

Overweight and obese adolescents, as well as adolescents with abdominal obesity, presented lower total energy intake, in addition to lower intake of sweets and pastries, fast food and sugar-sweetened beverages. In addition, fruit intake was positively associated with abdominal obesity, whereas sweets and pastries and fast-food were negatively associated with the same. These findings could be related to the bias in reporting food intake which has been previously described elsewhere (47). Indeed, it has been observed that obese adolescents under-report their food intake more than their lean counterparts (47, 48). Furthermore, foods that are more socially desirable and approved may be overestimated, and the opposite may also occur (47). On the other hand, these results may also be due to the cross-sectional design of this study, which might have distorted the temporal relationship between diet and weight. Adolescents who are overweight and obese probably decrease their intake of high-energy, dense foods, in order to lose weight (49).

In this study, various measures of obesity had differential associations with food group, physical activity and SES. Therefore, it may be important to use different measures of obesity, because the association of each measurement with health risks seems to be distinct. BMI is correlated with body fat, which, in excessive amounts, is related to metabolic complications, but BMI is also associated with lean mass, which may lead to some misclassification (50). There is evidence that WC is associated with visceral adipose tissue and is an independent risk factor for insulin resistance, hyperinsulinemia, dyslipidemia and hypertension in youth (21, 51, 52). On the other hand, WHtR has been shown to be superior in its ability to predict cardiovascular disease risk factors, compared with either BMI or percentage body fat in children (20, 53, 54).

Some limitations of the present study should be acknowledged. As in every cross-sectional study, conclusions related to cause and effect cannot be drawn. The measures of abdominal obesity used in this study are indirect estimates of abdominal fat, and there are some sophisticated methods of accurately measuring abdominal fat, such as magnetic resonance imagining and dual energy x-ray absorptiometric densitometry. However, such techniques cannot feasibly be applied in large epidemiological studies, because they are complex, time-consuming and expensive. Finally, with self-reported physical activity and dietary intake data, one cannot rule out some reporting bias, although both questionnaires have been previously tested (25, 29).

In conclusion, we found that higher milk and ready-to-eat cereal intake and father's employment status have a protective effect on abdominal obesity. In addition, we also reported that different measures of obesity have distinct associations with food group intake and SES. Thus, prospective and randomized clinical investigations are needed to examine the roles of food group intake, physical activity and SES in the development of obesity, as assessed by different measures.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Paper II

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ORIGINAL ARTICLE

Association between dairy product intake and abdominal obesity in Azorean adolescents

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BACKGROUND: Some studies have reported an inverse association between dairy product (DP) consumption and weight or fat mass loss.
OBJECTIVES: The objective of our study was to assess the association between DP intake and abdominal obesity (AO) among Azorean adolescents.
SUBJECTS/METHODS: This study was a cross-sectional analysis. A total of 903 adolescents (370 boys) aged 15–16 years was evaluated. Anthropometric measurements were collected (weight, height and waist circumference (WC)) and McCarthy's cut-points were used to categorize WC. AO was defined when WC was $\geqslant$90th percentile. Adolescent food intake was assessed using a self-administered semiquantitative food frequency questionnaire and DP intake was categorized in <2 and $\geqslant 2$ servings/day. Data were analyzed separately for girls and boys, and logistical regression was used to estimate the association between DPs and AO adjusting for potential confounders.
RESULTS: The prevalence of AO was 54.9% (boys: 32.1% and girls: 70.7%, $P<0.001$). For boys and girls, DP consumption was 2.3 ± 1.9 and 2.1 ± 1.6 servings/day ($P=0.185$), respectively. In both genders, the proportion of adolescents with WC $<$90th percentile was higher among individuals who reported a dairy intake of $\geqslant 2$ servings/day compared with those with an intake <2 servings/day (boys: 71% vs 65% and girls: 36% vs 24%, $P<0.05$). After adjustments for confounders, two or more DP servings per day were a negative predictor of AO (odds ratio, 0.217; 95% confidence interval, 0.075–0.633) only in boys.
CONCLUSION: We found a protective association between DP intake and AO only in boys.

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Keywords: dairy products; waist circumference; adolescents

INTRODUCTION

The prevalence of abdominal obesity (AO) among adolescents in developed countries has been increasing, as have associated complications.[1–4]

Primary preventions of obesity, as well as AO, should focus on energy balance, but intake of some foods has been shown to affect the development of AO among adolescents.[5–7] Recently, several[5,8–11] but not all[12,13] epidemiological studies about this topic have shown that dairy product (DP) intake is inversely related to AO and body fat in children and adolescents. DPs are an important source of nutrient and/or bioactive constituents such as calcium. It appears that a high-calcium diet may have a role in preventing fat accumulation by affecting adipocyte lipid metabolism, lipogenesis and lypolysis, fat oxidation and fat absorption, through genetic and non-genetic mechanisms.[14] Furthermore, according to some studies, calcium from a dairy source may be more effective than other dietary sources in reducing and regulating body fat.[15,16] Although, the mechanism of this DP 'anti-obesity' effect is not yet clear, it was suggested that it may be mediated by dairy components (e.g., whey protein) other than calcium.[17]

At the present time, consumption of milk and milk products is not considered by dietary recommendations to have a special role in weight control,[18] despite some evidence from epidemiological observational and experimental studies linking dairy food consumption to obesity.[7,11,13,15,19–23] Furthermore, studies examining the association between markers of AO–such as waist circumference (WC)–and DP intake among adolescents are scarce. Hence, the aim of the present study was to assess the association between DP intake and AO among Azorean adolescents.

MATERIALS AND METHODS

Sampling

Data for the present study were derived from a longitudinal school-based study–The Azorean Physical Activity and Health Study II, which aimed to evaluate physical activity, physical fitness, overweight/obesity prevalence, dietary intake, quality of life and other health-related factors. The study was carried out in six of the nine Azorean Islands (S Miguel, Terceira, Faial, Pico, S Jorge and Graciosa), where 95% of the Azorean population live.[24]

All participants in this study were informed of its goals, and the parent or guardian of each participant provided written informed consent. The study was approved by the Faculty of Sport and the Portuguese Foundation for Science and Technology Ethics Committees; it was conducted in accordance with the World Medical Association's Helsinki Declaration for Human Studies.

The population was selected by means of proportionate stratified random sampling, taking into account location (island) and number of students, by age and sex, in each school. The estimated number of subjects needed was 1422, but to prevent information loss, data were collected for

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1515 adolescents. For the purpose of the study and taking into account that cutoffs used to categorize WC are available only for age under 17, we only included adolescents aged 15-16 ($n = 960$), from which 57 were not included due to missing information on dietary intake ($n = 41$) and WC ($n = 16$). Therefore, this led a total of 903 participants (370 boys). Power analysis of the final sample was calculated *post hoc* being higher than 0.8 for P-value <0.05. Finally, the sample was weighted in accordance with the distribution of the Azorean population in schools and to guarantee the real representativeness of each group (by age and gender).

Anthropometric measures

Body height and body weight. Body height and body weight were determined using standard anthropometric methods. Height was measured to the nearest millimeter in bare or stocking feet, with adolescents standing upright against a Holtain portable stadiometer (Crymych, Pembrokeshire, UK). Weight was measured to the nearest 0.10 kg, with participants lightly dressed (underwear and t-shirt) and with the use of a portable digital beam scale (Tanita Inner Scan BC 532, Tokyo, Japan).

Body mass index (BMI) was calculated using the ratio of weight/height^2 (kg/m^2). Subjects were classified as normal weight, overweight or obese, according to age- and sex-specific cutoff points specified by the International Obesity Task Force.[25]

Waist circumference. WC measurements were taken midway between the tenth rib and the iliac crest and recorded to 0.1 cm. A non-elastic flexible tape measure was used, with subjects standing erect with arms by sides, feet together and abdomen relaxed, as well as without clothing covering the waist area. Subjects were divided into two categories ($<$90th and $\geqslant$90th percentiles) according to age- and sex-specific cutoff points specified by McCarthy.[26] Subjects who were in the 90th percentile or above were considered to have AO.[27]

Pubertal stage. To determine pubertal stage (ranging from stage 1 to 5), each subject was asked to self-assess his/her stage of secondary sex characteristics. Stage of breast development in girls and genital development in boys was evaluated according to criteria outlined by Tanner and Whitehouse.[28] For the purposes of the present analyses, the original 5-stage Tanner scale was collapsed into four categories: (i) stage 1 or 2; (ii) stage 3; (iii) stage 4; and (iv) stage 5.

Socio-demographic and lifestyle variables

Participants answered a questionnaire that assessed several socio-demographic and lifestyle variables.

Smoking. Participants were classified as non-smokers, former smokers (individuals who had stopped smoking for at least 6 months), occasional smokers (individuals who smoked, on average, less than one cigarette a day) and current smokers (individuals who smoked at least one cigarette a day).[29] Occasional smokers were recoded and combined with current smokers, due to their small sample size.

Parental education. For the present study, the highest level of parental education (measured by number of school years completed) was used as a proxy measure of socio-economic status. Participants were divided into three categories, reflecting divisions within the Portuguese educational system: mandatory or less ($\leqslant$9 school years), secondary (10-12 school years) and college/university ($>$12 school years).

Dietary intake

Dietary intake was measured via a self-administered semiquantitative food frequency questionnaire that covered the previous 12 months and included 86 food item and beverage categories, validated for Portuguese adolescents.[30] This semiquantitative food frequency questionnaire was designed in accordance with criteria laid out by Willett[31] and adapted to include a variety of typical Portuguese food items. For each item, the questionnaire offered nine frequency response options, ranging from 'never' to 'six or more times per day', and measured portion size and seasonality. Any foods not listed in the questionnaire could be listed by participants in a free-response section. Energy and nutritional intake were estimated with regard to respondents' ratings of the frequency, portion and seasonality of each item, using the software Food Processor Plus (ESHA Research Inc., Salem, OR, USA). This software uses nutritional information from the United States that has been adapted for use with typical Portuguese foods and beverages.

This study considered two DPs: milk and yogurt. We used the following amounts per servings: 250 ml for milk (whole, reduced fat and fat free) and 200 g for yogurt. Participants were categorized according to the new Portuguese Food Wheel guide as eating two or more servings of milk and yogurt per day or less ($\geqslant$2 and $<$2 servings/day).[32] Portions of cheese per day were considered separately–that is, one serving comprised 40 g of cheese (cottage and cream cheese). In view of the fact that there are no recommendations for portions of cheese, the median servings of cheese (median $=$ 0.3 serving/day) were used to categorize participants.

Calcium intake was expressed as the calcium-to-protein ratio, to offset the effects that these two nutrients have on each another and because ratio eliminates most of the portion size estimation error.[33,34]

Physical activity

Physical activity was assessed via a self-report questionnaire[35] that had been shown to have good test-retest reliability among Portuguese adolescents (intraclass correlation coefficient 0.92-0.96).[36] From this questionnaire, a summative index (range 5-20) was derived. Participants who reported $>$12.5 points were classified as active, and those who reported 12.5 points or less comprised the low-activity group.

Statistical analysis

Data was analyzed separately for girls and boys, because it was believed that sex might influence the accumulation of abdominal fat.[37] Descriptive analysis showed lifestyle, socio-demographic, anthropometric and dietary characteristics of the sample by gender. All variables were tested for normality. Independent sample *t*-tests were performed to compare continuous variables, and the χ^2-test was used with categorical variables. The Mann-Whitney test was performed for quantitative variables when appropriate.

The association between DP intake and WC (the dependent variable) was investigated using logistical regression (multivariate models). The multivariate-adjusted models were controlled for the following potential confounders: age (in years), BMI (in kg/m^2), pubertal stage (reference–stage 1 or 2), physical activity level (reference–low activity), energy intake (in kcal), calcium-to-protein ratio and dietary fiber (in g/1000 kcal). Age, BMI, energy intake, calcium-to-protein ratio and dietary fiber were entered as continuous variables. To control for possible confounding by cheese intake, we also adjusted the multivariate models for this variable (reference–$<$0.3 serving/day). An odds ratio (OR) and a 95% confidence interval (CI) were computed for the $\geqslant$2 servings/day DPs intake category.

A P-value of <0.05 was regarded as significant. All analyses were performed using the PASW Statistic v.18 (SPSS, Chicago, IL, USA).

RESULTS

The mean age was 15.5 ± 0.5 years for both genders ($P = 0.695$). The prevalence of overweight/obesity was 32.2% (boys: 29.3% and girls: 34.2%, $P = 0.129$) and of AO was 54.9% (boys: 32.1 and girls: 70.7%, $P < 0.001$). Descriptive data on WC and other covariates are presented in Table 1. Average BMI for both genders was higher in those who had AO (boys: 26.4 ± 4.0 kg/m^2 vs 20.7 ± 2.2 kg/m^2, $P < 0.001$; girls: 23.7 ± 3.6 kg/m^2 vs 20.4 ± 2.9 kg/m^2, $P < 0.001$), while no significant association was found between boys and girls. However, the percentage of adolescents who had AO was higher in girls (70.7% vs 32.2%, $P < 0.001$). Boys were more active than girls (83.7% vs 52.3%, $P < 0.001$). Boys who had AO were less active than those with WC $<$90th percentile (77.3% vs 86.5%, $P = 0.027$).

Table 1. Characteristics of the subjects by gender

	Boys			*Girls*			*Boys*	*Girls*	P
	WC		P[a,b]	*WC*		P[a,b]	*Total (n = 370)*	*Total (n = 533)*	
	<P90 (n = 251)	≥P90 (n = 119)		<P90 (n = 156)	≥P90 (n = 377)				
BMI[c] (kg/m²)	20.7 ± 2.2	26.4 ± 4.0	<0.001	20.4 ± 2.9	23.7 ± 3.6	<0.001	22.5 ± 3.9	22.7 ± 3.7	0.119
Parental education (%)									
Mandatory or less	52.2	53.4		51.9	59.6		52.6	57.3	
Secondary	29.3	33.1	0.465	29.2	23.5	0.252	30.5	25.1	0.213
College/university	18.5	13.6		18.8	17.0		16.9	17.5	
Smoking status (%)									
Non-smoker	82.1	86.4		81.1	81.5		83.5	83.7	
Former smoker	5.2	3.4	0.552	2.6	5.6	0.086	4.6	4.7	0.944
Current smoker	12.7	10.2		8.3	13.0		11.9	11.6	
Pubertal stage (%)									
Stage 1 or 2	1.2	2.5		1.9	0.5		1.6	0.9	
Stage 3	9.6	16.0	0.017	24.5	20.4	0.009	21.6	21.6	<0.001
Stage 4	64.1	47.1		63.2	57.3		59.4	59.0	
Stage 5	25.1	34.5		10.3	21.8		28.1	18.4	
Physical activity (%)									
Low active	13.5	22.7	0.027	48.1	47.7	0.944	16.5	47.8	<0.001
Active	86.5	77.3		51.9	52.3		83.5	52.2	

Abbreviations: BMI, body mass index; WC, waist circumference; P90, 90th percentile. [a]Analysis by Student's *t*-test for continuous variables. [b]Analysis by χ^2 for categorical variables. [c]Mean ± s.d.

Energy intake and dietary characteristics for each gender are presented in Table 2. Boys' diets were higher in energy and total and saturated fats, and lower in dietary fiber and carbohydrates, compared with girls ($P<0.05$ for all). There was no significant difference between boys and girls with regard to dairy, calcium and protein intake.

Distribution of adolescents by DP intake category was based on WC, as indicated in Figure 1. In both genders, the proportion of adolescents with WC <90th percentile was higher among individuals who had an intake of ≥2 dairy servings/day, compared with those who had an intake <2 servings/day (boys: 71% vs 65% and girls: 36% vs 24%, $P<0.05$, respectively).

Multivariate-adjusted ORs for AO for DP intake are shown in Table 3. In both genders, after adjusting for demographic variables and physical activity level, intake of ≥2 dairy servings per day was a negative predictor of AO (boys: OR, 0.474, 95% CI, 0.238–0.945 and girls: OR, 0.545, 95% CI, 0.355–0.839). After adjusting also for dietary factors (model 2), only boys who had ≥2 servings of DPs per day had lower risk of AO (boys: OR, 0.217, 95% CI, 0.075–0.633 and girls: OR, 0.560, 95% CI, 0.307–1.022). A similar result was observed after adjustment for cheese intake (model 3).

DISCUSSION

In this study, we observed a protective association between eating two or more servings of milk and yogurt, and AO in boys. This association was not confounded by other lifestyle factors or nutritional variables, particularly calcium intake.

Evidence from observational studies on the relationship between consumption of DPs and AO in adolescents are limited, and most of them[8,11,12,38,39] have focused on weight and body fat loss. In children and adolescents, few studies have reported a protective association between dairy consumption and overweight/obesity. For instance, Moore *et al.*[11] in a cross-sectional study, found that adolescents in the lowest category of total dairy intake had higher BMIs and more subcutaneous fat. However, longitudinal studies have yielded conflicting results. Of 10 prospective cohort studies with children and adolescents reviewed by Louie,[40] 6 reported no significant association and 1 reported an increased risk, while 3 found a protective association between dairy consumption and the risk of being overweight/obese.

Regarding the association between dairy consumption and AO, one study with adolescent found that central body fat measures (i.e., WC and the sum of suprailiac and scapular skinfold thicknesses) were inversely associated with total dairy intake.[5] In adults, this relationship was examined in two cross-sectional studies.[41,42] However, in only one study[41] an inverse association was shown between dairy intake (milk and yogurt) and AO. In two longitudinal studies,[22,37] DPs were found to be negatively associated with WC. Vergnaud *et al.*[22] reported that milk and yogurt intake were protective against 6-year changes in WC only in men who were initially overweight, and Halkjaer *et al.*[37] found a protective association only in women, with a 5-year difference in WC for high-fat dairy intake.

The literature suggested that when DPs were separated into subgroups (i.e., milk, yogurt and cheese), a significant association with AO was only seen for some.[14,40] To the best of our knowledge, only one study investigated the cross-sectional relationship between 'type' of dairy and AO in adolescents. Bradlee *et al.*,[5] in data from the Third National Health and Nutrition Examination Surveys (NHANES III and NHANES 1998–2000), showed that adolescents who met the criteria for AO reported consuming significantly fewer milk servings per day. However,

Table 2. Dietary characteristics of the subjects by gender

	Boys			*Girls*			*Boys*	*Girls*	P
	WC		P	*WC*		P	*Total* (n = *370)*	*Total* (n = *533)*	
	<*P90* (n = *251)*	≥*P90* (n = *119)*		<*P90* (n = *156)*	≥*P90* (n = *377)*				
Energy intake[a,b] (kcal)	2720 ± 416.5	2631.0 ± 404.9	0.454	2544.9 ± 454.3	2362.2 ± 278.4	0.087	2691.9 ± 411.5	2415.6 ± 333.4	< 0.001
Carbohydrates[a,b] (% of energy)	49.7 ± 8.3	50.2 ± 9.2	0.618	51.6 ± 8.6	50.7 ± 7.8	0.246	49.9 ± 8.6	51.0 ± 8.0	0.045
Total fat[a,b] (% of energy)	33.5 ± 5.2	33.2 ± 6.1	0.647	32.4 ± 6.0	32.6 ± 5.3	0.755	33.4 ± 5.5	32.5 ± 5.5	0.025
Saturated fat[a,b] (% of energy)	12.1 ± 2.6	11.6 ± 2.5	0.157	11.4 ± 2.6	11.2 ± 2.3	0.528	11.9 ± 2.6	11.3 ± 2.4	< 0.001
Protein[a,b] (% of energy)	17.9 ± 3.6	17.9 ± 4.1	0.869	17.5 ± 3.2	18.1 ± 3.8	0.131	17.9 ± 3.8	18.0 ± 3.6	0.576
Dietary fiber[a,b] (g/1000 kcal)	9.1 ± 3.0	9.5 ± 3.4	0.395	10.6 ± 4.2	10.5 ± 3.4	0.797	9.2 ± 3.1	10.5 ± 3.7	< 0.001
Total calcium intake[a,c] (mg)	1303.6 ± 791.9	1328.2 ± 811.5	0.874	1317.7 ± 742.8	1169 ± 680.7	0.017	1311.5 ± 797.0	1215.2 ± 702.8	0.086
Calcium-to-protein ratio[a,b]	11.1 ± 3.7	11.2 ± 4.4	0.830	12.0 ± 3.6	11.5 ± 4.0	0.130	11.2 ± 3.9	11.6 ± 3.8	0.048
Dairy intake[a,c] (servings/day)	2.4 ± 1.8	2.3 ± 2.1	0.289	2.3 ± 1.6	2.0 ± 1.6	0.058	2.3 ± 1.9	2.1 ± 1.6	0.185
Cheese[a,c] *(servings/day) (%)*	0.5 ± 0.7	0.5 ± 0.7	0.793	0.5 ± 0.8	0.4 ± 0.6	0.996	0.52 ± 0.67	0.46 ± 0.66	0.048
< 0.3 serving/day	57.9	59.7	0.753	71.0	65.8	0.247	58.5	67.3	0.007
≥ 0.3 serving/day	42.1	40.3		29.0	34.2		41.1	32.7	

Abbreviations: WC, waist circumference; P90, 90th percentile. [a]Mean ± s.d. [b]Between-gender and WC percentiles analysis by Student's *t-test*. [c]Between-gender and WC percentiles analysis by Mann-Whitney test.

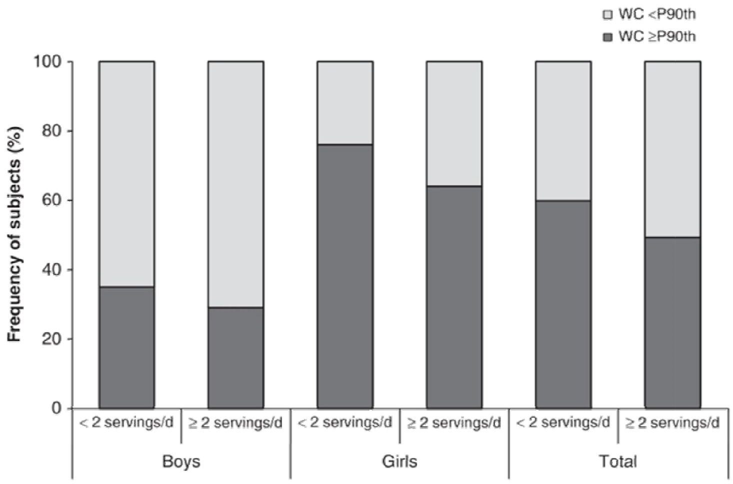

Figure 1. Distribution of subjects in dairy intake categories based on their WC.

it is noteworthy that the same finding for cheese consumption was only observed in NHANES III data. Regarding the beneficial role of different DPs on body fat, in children and adolescents, cross-sectional[11,43] and longitudinal[8,10,39] studies have shown an inverse association between milk and adiposity, but this association was not found in other studies.[12,38] On the other hand, cheese consumption was only addressed in adults,[21,41,42] and some of these studies[41,42] reported a positive association with prevalence of obesity and AO. Moreover, an inverse association between yogurt intake and adiposity was only shown in Beydoun's study.[41] Therefore, overall, the evidence suggests that milk and yogurt are associated with lower adiposity, with cheese having the opposite effect.[14] In this study, we only analyzed the association between total milk and yogurt intake and AO (milk and yogurt when considered separately were not associated with AO; data not shown).

In agreement with our results, other studies have found a higher proportion of AO in girls.[1,2] Furthermore, the prevalence of

Table 3. Odds ratios (and 95% CIs) for the relationships between dairy servings intake (⩾2 servings/day) and abdominal obesity

	Boys (n = *370*)			*Girls* (n = *533*)		
	OR	*95% CI*	P	*OR*	*95% CI*	P
Model 1	0.474	0.238 - 0.945	0.034	0.545	0.355 - 0.839	0.006
Model 2[a]	0.217	0.075 - 0.633	0.005	0.560	0.307 - 1.022	0.059
Model 3[b]	0.208	0.070 - 0.617	0.005	0.563	0.307 - 1.032	0.063

Abbreviations: BMI, body mass index; CI, confidence interval; OR, odds ratio; PA, physical activity. All models were adjusted for age (years), BMI (kg/m^2), pubertal stage and PA level. [a]Model 2 was also adjusted for energy intake (kcal), dietary fiber (g/100 kcal) and calcium-to-protein ratio. [b]Model 3 was also adjusted for energy intake (kcal), dietary fiber (g/100 kcal), calcium-to-protein ratio and cheese intake (reference– < 0.3 serving/day).

AO has been increasing at a faster rate in girls than in boys.[1,2] On the other hand, even after adjusting for confounders, there was a non-significant association between dairy consumption and AO in girls. There are several possible explanations for these findings. WC is unlikely to be due to visceral adipose tissue alone; it probably reflects both visceral and subcutaneous fat. It is known that adult women have a greater amount of subcutaneous fat than men and less visceral fat, a difference closely related to gender differences in cardiometabolic disease risk.[44-46] These differences begin early in life and become more apparent in puberty due to changes in sex hormone levels. However, studies on gender differences in visceral fat in adolescents have discrepant results. Some have suggested that boys have more visceral fat than girls,[47,48] while others have found no significant differences.[49] Nevertheless, it is important to note that this is one of few studies that addressed sexual maturation, controlling for the extent of biological growth and the individual nutritional needs of adolescents.[50]

Several studies have suggested a beneficial effect of some dairy components, especially calcium, on weight and body fat loss. The plausible mechanism most frequently cited relates to the effects of calcium on adipocyte metabolism and fatty acid absorption from the gastrointestinal tract.[14] Zemel[51,52] has demonstrated that the concentration of intracellular Ca^{2+} in human adipocyte is increased by the stimulation of parathyroid hormone and 1,25-dihydroxyvitamin D_3, which occurs in response to a low-calcium diet. The resultant increase in intracellular Ca^{2+} exerts a coordinated effect on adipocyte lipid metabolism, serving to stimulate lipogenic gene expression and lipogenesis, thereby increasing lipid filling and adiposity. Calcium is also able to increase fecal excretion of fat via the formation of insoluble fatty acid soaps or by binding bile acids.[14] However, these mechanisms do not entirely explain the observed 'antiobesity' effects of DPs.[53] Dairy is also an important source of proteins, and whey proteins have been positively associated with satiety.[14] Moreover, it has been shown that whey protein inhibits angiotensin-converting enzyme and consequently inhibits the production of angiotensin II hormone,[53] which has been reported to upregulate adipocyte lipogenesis, resulting in the inhibition of fat deposition.[54] Yet, there is lack of knowledge concerning the mechanisms that explain the effects of DPs on the loss of central adiposity. Visceral adipose tissue has greater amounts of 11-β-HSD-1 (11-β-hidroxysteroid dehydrogenase), which can generate active cortisol from cortisone, than does subcutaneous adipose tissue.[55] *In-vitro* data obtained from mice suggest that the selective overexpression of 11-β-HSD-1 in adipose tissue results in central obesity.[56] It has been suggested that a high-calcium and high-dairy diet may reduce cortisol production because visceral adipocytes stimulate lipolysis.[55] Nevertheless, no studies have specifically examined these mechanisms in children and adolescents, and the dynamic metabolic changes that occur during growth and puberty may further complicate these issues.[57] Furthermore, it is possible that the interaction between body fat and DPs depends not only on the threshold of percentage of body fat,[9] but also indeed on its distribution by gender, with boys having more visceral fat. However, DP consumption may also be associated with dimensions of a healthier lifestyle that protect against adiposity, such as frequency of eating episodes.[58,59]

There are some limitations in our study that should be addressed. First, the cross-sectional design of this study prevents any conclusions related to cause and effect. Second, we did not include cheese in DPs, because the food frequency questionnaire was not specifically designed to account for different types of cheese, but we did control for the effects of cheese intake. Third, the measure of AO used in this study is an indirect estimate of abdominal fat. However, WC has been used as a simple anthropometric tool, to access and identify adolescents who are at risk of having AO. It has been suggested that WC is one of the most common proxy measures of AO[1] and is strongly associated with visceral adipose tissue.[60]

In conclusion, we found that DPs have a protective effect on AO only in boys. These results suggest that DP intake may be related to body fat distribution. Future research, with more accurate measures of visceral adiposity, is needed to address this relationship and to examine the effects of different types of DPs.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Paper III

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ORIGINAL ARTICLE

Milk intake is inversely related to body mass index and body fat in girls

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Abstract Dairy foods comprise a range of products with varying nutritional content. The intake of dairy products (DPs) has been shown to have beneficial effects on body weight and body fat. This study aimed to examine the independent association between DP intake, body mass index (BMI), and percentage body fat (%BF) in adolescents. A cross-sectional, school-based study was conducted with 1,001 adolescents (418 boys), ages 15–18 years, from the Azorean Archipelago, Portugal. Anthropometric measurements were recorded (weight and height), and %BF was assessed using bioelectric impedance analysis. Adolescent food intake was measured using a self-administered, semi-quantitative food frequency questionnaire. Data were analyzed separately for girls and boys, and separate multiple linear regression analysis was used to estimate the association between total DP, milk, yogurt, and cheese intake, BMI, and %BF, adjusting for potential confounders. For boys and girls, respectively, total DP consumption was 2.6±1.9 and 2.9±2.5 servings/day (P=0.004), while milk consumption was 1.7±1.4 and 2.0±1.7 servings/day (P=0.001), yogurt consumption was 0.5±0.6 and 0.4±0.7 servings/day (P=0.247), and cheese consumption was 0.4±0.6 and 0.5 ±0.8 servings/day (P=0.081). After adjusting for age, birth weight, energy intake, protein, total fat, sugar, dietary fiber, total calcium intake, low-energy reporters, parental education, pubertal stage, and physical activity, only milk intake was negatively associated with BMI and %BF in girls (respectively, girls: β=−0.167, P=0.013; boys: β=−0.019, P=0.824 and girls: β=−0.143, P=0.030; boys: β=−0.051, P=0.548). *Conclusion*: We found an inverse association between milk intake and both BMI and %BF only in girls.

Keywords Dairy products · Milk · Body mass index · Body fat · Adolescents

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Introduction

The increasing prevalence of overweight and obesity among children and adolescents has been described in developed and developing countries alike [60]. In the European Union, the prevalence of childhood overweight and obesity is approximately 25 %, with Southern European countries showing the highest prevalence rates [25, 57]. Indeed, in Portugal, 21.6 % of girls and 23.5 % of boys, ages 10 to 18 years, are overweight or obese [44]. Furthermore, according to some studies, more than 60 % of overweight/obese adolescents tend be obese as adults [19, 39]. Obesity also has profound public health implications, being associated with increased risk of such chronic conditions as diabetes and cardiovascular disease and with high health care costs [34].

Obesity is a complex, multifactorial disease; however, the primary cause is related to the energy imbalance that results from low physical activity (PA) and inadequate nutrition [14, 29]. In recent years, increasing attention has been focused on the preventive effects of dairy product (DP) intake. However, there is conflicting evidence regarding the relationship between DP intake and obesity. Some observational studies of children and adolescents have reported a significant inverse relationship between dairy consumption and measures of body composition [1, 17, 31, 37, 38], while others have found no association [2, 23, 36]. Several mechanisms have been proposed to explain how DP intake influences fatness and body composition. Although calcium was mentioned as a principal bioactive component of DPs, with effects on adipocyte lipid metabolism, other constituents such as proteins (in particular, whey proteins) and their peptide derivatives may affect body weight by regulating food intake and appetite [16].

The purpose of this study was to examine the independent association between DP intake, body mass index (BMI), and percentage body fat (%BF) after taking into account dietary factors, physical activity level (PAL), and other potentially confounding variables in a sample of Portuguese adolescents.

Materials and methods

Sampling

Data for the present cross-sectional study were derived from a longitudinal school-based study—The Azorean Physical Activity and Health Study II, which aimed to evaluate PA, physical fitness, overweight/obesity prevalence, dietary intake, health-related quality of life, and related factors in 15- to 18-year-old adolescents. This study was carried out in six of the nine Azorean Islands (S. Miguel, Terceira, Faial, Pico, S. Jorge, and Graciosa), where 95 % of the Azorean population lives [24].

All participants in this study were informed of its goals, and the parent or guardian of each participant provided written informed consent. The study was approved by the Faculty of Sport, University of Porto and the Portuguese Foundation for Science and Technology Ethics Committee; it was conducted in accordance with the World Medical Association's Helsinki Declaration for Human Studies.

The population was selected by means of proportionate stratified random sampling, taking into account location (island) and number of students, by age and sex, in each school. The estimated number of subjects needed was 1,422, but in order to prevent information loss, data were collected for 1,515 adolescents. Some adolescents were not included in our analysis (n=514) because information was missing on their dietary intake (n=286), BMI (n=15), %BF (n=15), and birth weight (n=198). This resulted in a collection of data for a total of 1,001 participants (418 boys). The subjects who were excluded from this study did not significantly differ from those who were included, with regard to age (16.2±1.0 vs. 16.1±1.0 years, P=0.118), parental education (8.8±4.4 vs. 9.2±4.4 years, P=0.219), gender (girls, 60.3 vs. 58.2 % and boys, 39.7 vs. 41.8 %, P=0.471), and BMI (22.9±4.1 vs. 22.9±3.9 kg/m^2, P=0.888). Finally, the sample was weighted, so as to balance it in accordance with the distribution of the Azorean population in schools and to guarantee the real representativeness of each group (age and gender).

Anthropometric measures

Body height and body weight were determined using standard anthropometric methods. Height was measured to the nearest millimeters in bare or stocking feet, with adolescents standing upright against a Holtain portable stadiometer (Crymych, Pembrokeshire, UK). Weight was measured to the nearest 0.10 kg, with participants lightly dressed (underwear and T-shirt) and with the use of a portable digital beam scale (Tanita Inner Scan BC 532, Tokyo, Japan).

BMI was calculated using the ratio of weight/height^2 (in kilograms per square meter). Subjects were classified as normal weight, overweight, or obese, according to age- and sex-specific cutoff points specified by the International Obesity Task Force [10, 11]. Underweight subjects (2.8 %) were combined with normal weight subjects due to the fact that they represented a small proportion of the sample. %BF was assessed using bioelectric impedance analysis–BIA (Tanita Inner Scan BC 532, Tokyo, Japan).

Pubertal stage

To determine pubertal stage (which ranged from 1 to 5), each subject was asked to self-assess his/her stage of development of secondary sex characteristics. Breast development in girls and genital development in boys was evaluated according to criteria outlined by Tanner and Whitehouse [50].

Sociodemographic and lifestyle variables

Participants answered a questionnaire that assessed several sociodemographic and lifestyle variables.

Smoking

Participants were classified as nonsmokers, former smokers (individuals who had stopped smoking for at least 6 months), occasional smokers (individuals who smoked, on average, less than one cigarette a day), and current smokers (individuals who smoked at least one cigarette a day) [59]. Occasional smokers were recoded and combined with current smokers due to the fact that they represented a small proportion of the sample.

Parental education

For the present study, the highest level of parental education (measured by the number of school years completed) was used as a proxy measure of socioeconomic status. Participants were divided into three categories, reflecting divisions within the Portuguese educational system: mandatory or less (≤9 school years), secondary (10 to 12 school years), and college/university.

Dietary intake

Dietary intake was measured via a self-administered semi-quantitative food frequency questionnaire (FFQ) that covered the previous 12 months and included 86 food item and beverage categories, validated for Portuguese adolescents [41]. This semiquantitative FFQ was designed in accordance with criteria laid out by Willett et al. [58] and adapted to include a variety of typical Portuguese food items. For each item, the questionnaire offered nine frequency response options, ranging from "never" to "six or more times per day," and measured portion size and seasonality. Any foods not listed in the questionnaire could be listed by participants in a free-response section. Energy and nutritional intake were estimated with regard to respondents' ratings of the frequency, portion, and seasonality of each item, using the software Food Processor Plus (ESHA Research Inc., Salem, OR, USA). This program uses nutritional information from the USA that has been adapted for use with typical Portuguese foods and beverages.

DPs were defined according to the new Portuguese food wheel guide [42]. The amounts of milk (whole, reduced-fat, and fat-free), yogurt, and cheese (cottage and cream cheese) that counted as single servings were considered to be 250 ml, 200 g, and 40 g, respectively.

Physical activity

PA was assessed via a self-report questionnaire that evaluated leisure times physical activities [52]. This questionnaire has been shown to have good test–retest reliability among Portuguese adolescents (intraclass correlation coefficient, 0.92–0.96) [33]. From this questionnaire, a summative index (range, 5–20) was derived. Participants whose scores were greater than 12.5 points were classified as active, while those whose scores were 12.5 points or less comprised the low-activity group.

Statistical analysis

Data were analyzed separately for girls and boys. The Kolmogorov–Smirnov test was used to assess the assumption of normality. Independent sample *t* test or Mann–Whitney test were performed to compare continuous variables between gender, and the chi-square test was used with categorical variables. In this report, descriptive analysis is presented in terms of means and standard deviations.

To access associations between DP and BMI and %BF, we performed separate regression linear models for each DP: total dairy, milk, yogurt, and cheese. For each DP, we adjusted linear regression model for age (in years), birth weight (in kilograms), energy intake (in kilocalories), total calcium intake (in milligrams), protein intake (in grams per kilogram of weight), sugar intake (in percent of energy), total fat intake (in percent of energy), pubertal stage, PAL, and parental education. Age, birth weight, and dietary variables were entered as continuous variables. Pubertal stage, PAL, and parental education were entered as dummy variables. Furthermore, we adjusted linear models by misreporting of energy intake that was estimated using the ratio between reported energy intake and predicted basal metabolic rate (EI/BMR) [4, 5, 18]. In this study, a mean PAL of 1.73 was assumed for female adolescents ages 15 to 17 years old, 1.75 for boys ages 15 to 17 years old, and 1.70 and 1.85 for girls and boys age 18 years old, respectively [6, 7]. The thresholds that defined low-energy reporters (under-reporters) were 1.70 and 1.71 for girls and boys between 15 and 17 years old and 1.67 and 1.81 for girls and boys age 18 years. "Low-energy reporter" (a categorical variable) was included in the model as a confounding factor.

A P value of <0.05 was regarded as significant. All analyses were performed using PASW Statistic v.18 (SPSS, Chicago, IL, USA).

Results

Descriptive characteristics of the adolescents in the sample are shown in Table 1. The prevalence of overweight/obesity was 29.2 % in boys and 32.6 % in girls (P=0.252). In our study, boys were more active than girls (respectively, 83.7 vs. 50.4 %, $P<0.001$). No significant difference was seen in the BMI and birth weight of boys compared to girls ($P>$ 0.05, for all). In this study, for Tanner stages, 0.7 % of girls were reported to be in stage 2, 18.9 % in stage 3, 58.7 % in stage 4, and 21.7 % in stage 5. Similar figures for boys were as follows: 0.2 % in stage 1, 1.2 % in stage 2, 11.2 % in stage 3, 57.7 % in stage 4, and 30.2 % in stage 5.

Energy intake and dietary characteristics for each gender are presented in Table 2. Boys' diets were higher in energy, total fat, total dairy, milk, and calcium intake and lower in

Table 1 Characteristics of the study sample, by gender

	Girls (n=583)	Boys (n=418)	P value
Age[a,b] (years)	16.1±0.96	16.1±0.99	0.524
Weight[a,c] (kg)	58.9±10.9	67.8±12.9	<0.001
Height[a,c] (m)	1.60±0.06	1.72±0.07	<0.001
BMI[a,c] (kg/m^2)	22.9±3.8	22.9±4.0	0.983
BMI[d] (%)			0.252
Normal	67.4	70.8	
Overweight/obese	32.6	69.1	
Body fat[a] (%)	26.3±6.7	14.7±6.8	<0.001
Birth weight[a,c] (kg)	3.253±0.564	3.302±0.607	0.191
Parental education[d] (%)			0.518
Mandatory or less	50.2	46.5	
Secondary	34.8	37.6	
College/university	15.0	15.9	
Smoking status[d] (%)			0.564
Nonsmoker	88.3	86.1	
Former smoker	5.0	6.2	
Occasional/current smoker	6.7	7.7	
PA[d] (%)			<0.001
Low-active (≤12.5 points)	49.6	16.3	
Active (>12.5 points)	50.4	83.7	

BMI body mass index

[a] Mean±standard deviation

[b] Between-gender analysis by Mann–Whitney test

[c] Analysis by Student's*t* test for continuous variables

[d] Analysis by chi-square test for categorical variables

Table 2 Dietary characteristics of the study sample, by gender

	Girls (n=583)	Boys (n=418)	P value
Energy intake[a] (kcal)	2,273.9± 936.1	2,527.5± 1,078.6	<0.001
Protein[a] (% of energy)	18.0±3.7	18.2±4.0	0.578
Protein[b] (g/kg body weight)	2.0±1.6	2.0±1.7	0.824
Carbohydrates[b] (% of energy)	50.9±8.4	49.3±8.5	0.001
Sugar[b,c] (% of energy)	25.5±7.9	24.9±8.1	0.234
Total fat[b] (% of energy)	32.5±5.9	33.3±5.5	0.037
Dietary fiber[d] (g/1,000 kcal)	10.5±3.8	9.3±3.2	<0.001
Total calcium intake[d] (mg)	1166.0± 616.7	1260.6±707.9	<0.001
Total dairy[b] (servings/day)	2.6±1.9	2.9±2.5	0.004
Milk[b] (servings/day)	1.7±1.4	2.0±1.7	0.001
Yogurt[b] (servings/day)	0.5±0.6	0.4±0.7	0.247
Cheese[b] (servings/day)	0.5±0.6	0.5±0.8	0.081

Data are presented as the mean±standard deviation

[a] Between-gender analysis by Mann–Whitney test

[b] Between-gender analysis by Student's*t* test

[c] Sugars refer to all monosaccharides and disaccharides added to foods by the manufacturer, cook, or consumer, plus sugar naturally present in honey, syrups, and fruit juices

carbohydrates and dietary fiber, compared to girls ($P<0.05$, for all). There was no significant difference between boys and girls with regard to protein, sugar, yogurt, and cheese intake.

The results of the linear regression analyses used to estimate the association between DP intake and both BMI and %BF are presented in Table 3. After adjusting for age, birth weight, pubertal stage, dietary factors, parental education, and PAL (model 2), linear regression showed that milk intake was negatively and significantly associated with BMI and %BF only in girls (respectively, girls: $\beta=-0.167$, $P=0.013$; boys: $\beta=-0.019$, $P=0.824$ and girls: $\beta=-0.143$, $P=0.030$; boys: $\beta=-0.051$, $P=0.548$).

Discussion

The present study explored the relationship between DP (total, milk, yogurt, and cheese) intake and BMI and %BF among adolescents. The results suggested that intake of milk was negatively associated with BMI and %BF only in girls. The associations identified were not confounded by other lifestyle factors or dietary variables, particularly calcium intake.

The literature related to DP intake, especially different types of dairy (such as milk, yogurt, and cheese) and overweight/obesity in adolescents is limited and conflicting. Data from cross-sectional epidemiological studies support the hypothesis that milk and/or dairy consumption is associated with lower body fat and BMI in children and adolescents [1, 17, 31, 36, 37]. Moore et al. [31] found that adolescents in the lowest category of total dairy intake had higher BMIs and more subcutaneous fat in their subscapular and triceps skinfolds. However, in their analysis, this association was not explored with different types of DP, as were the associations in our study. In addition, Barba et al. [1] found a significant inverse association between frequency of milk consumption and BMI in children. In adults, a cross-sectional survey carried out with a large sample of the Portuguese population showed that milk intake was inversely related to BMI in men and premenopausal women [30].

Nevertheless, prospective studies have yielded inconsistent results. Johnson et al. [26] found that each serving of milk at 5 and 7 years of age was associated with a decrease in %BF at 9 years of age. A recent study also found that higher intake of whole milk at 2 years of age was associated with a decrement in BMI *z*-score at 3 years of age; however, when analysis was restricted to children with normal BMI (5th to <85th percentile for age), this association disappeared [23]. One study

Table 3 Association between DP intake and both BMI and %BF, using separate linear regression models for total dairy, milk, yogurt, and cheese intakes (servings/day), by gender

	BMI (kg/m²)						BF (%)					
	Girls (n=583)			Boys (n=418)			Girls (n=583)			Boys (n=418)		
	β	95 % CI	*P* value	β	95 % CI	*P* value	β	95 % CI	*P* value	β	95 % CI	*P* value
Model 1												
Total dairy	−0.065	−0.245, 0.004	0.057	−0.008	−0.150, 0.124	0.854	−0.055	−0.409, 0.041	0.109	0.008	−0.213, 0.262	0.837
Milk	−0.092	−0.406, −0.065	0.007	−0.033	−0.272, 0.114	0.421	−0.074	−0.651, −0.033	0.030	−0.025	−0.436, 0.232	0.547
Yogurt	−0.002	−0.416. 0.395	0.960	−0.023	−0.659, 0.371	0.582	−0.014	−0.881, 0.581	0.687	0.018	−1.087, 0.695	0.666
Cheese	−0.004	−0.511, 0.465	0.926	0.010	−0.449, 0.553	0.858	0.010	−0.567, 0.776	0.760	0.090	0.083, 1.467	0.028
Model 2												
Total dairy	−0.213	−0.913, 0.100	0.113	−0.021	−0.512, 0.444	0.890	−0.171	−1.498, 0.302	0.192	0.082	−0.607, 1.063	0.591
Milk	−0.167	−0.770, −0.089	0.013	−0.019	−0.421, 0.335	0.824	−0.143	−1.245, −0.062	0.030	−0.051	−0.862, 0.458	0.548
Yogurt	0.056	−0.246, 0.976	0.241	0.042	−0.437, 0.952	0.467	0.035	−0.654, 1.470	0.451	0.026	−0.935, 1.493	0.652
Cheese	0.055	−0.206, 0.870	0.226	−0.029	−0.772, 0.476	0.641	0.062	−0.274, 1.594	0.166	0.080	−0.374, 1.803	0.198

Model 1 is the unadjusted model. Model 2 is adjusted for age (years), birth weight (kilograms), energy intake (kilocalories), protein intake (grams per kilogram body weight), total fat intake (percent of energy), sugar intake (percent of energy), total calcium intake (milligrams), parental education, low-energy reporters, pubertal stage, and PAL

BMI body mass index, *BF* body fat, *CI* confidence interval

found that children who reported higher total milk intake experienced larger BMI gains, although this appeared to be mediated by energy intake [2]. However, this association was stronger for skim and 1 % milk intake than for whole or 2 % milk. In our study, we did not separate high-fat milk from low-fat milk, but we did control for the effects of fat intake.

Controlled intervention studies have also examined the relationship between dairy and/or milk consumption and fatness or body weight. Chan et al. [9] reported that, when 50 children with low calcium intake (<800 mg daily) were allocated to either a dairy-supplemented group or a control group for 6 months, children in the control group gained body fat during the study, while children in the dairy group had no significant change in body fat. On the other hand, in a randomized controlled trial that evaluated whether high milk (4 servings/day) consumption leads to greater weight loss in overweight children than low milk (1 serving/day) consumption during the course of a 16-week healthy-eating diet, St-Onge et al. [48] observed no significant differences between the groups in weight loss. Numerous intervention trials have been conducted in adults, and their findings are also conflicting. Of 11 studies without energy restrictions that were reviewed by Dougkas et al. [16], 7 reported no significant difference in weight or body fat with milk supplementation or dairy treatment, 2 reported weight gain, and 2 found higher body fat loss in groups with dairy-rich diets.

Our results showed no significant association between yogurt and cheese intake and BMI and %BF. In the literature, the majority of studies have examined milk intake, while only a few have used other dairy foods as the exposure variable. The weight of evidence suggests that milk intake is more likely to be associated with beneficial weight and body fat outcome [1, 26, 28, 38, 48], while a very small number of studies, conducted only with adults, have shown the beneficial effects of such other DPs as yogurt and cheese [3, 46, 56].

Discrepancies in the findings of existing studies could be due to differences in study design, methods for assessing diet and body composition (dual-energy X-ray absorptiometry [DXA] vs. BIA), treatment of milk and dairy intake (servings/day, grams, consumers vs. nonconsumers), adjustment of potentially confounding factors during analysis and/or due to the complexity of interactions between nutrients in humans [21, 36]. The inclusion of misreported dietary intakes may also lead to inconsistent findings; indeed, such misreporting is common in dietary studies [8, 20, 22]. Huang et al. [20] noted that underreporting is a major problem with adolescents, whereas overreporting is a significant problem with children under 12 years of age. In addition, Ventura et al. [55] found that under-reporters were selective in their underreporting, reporting fewer servings from the grain, dairy, sweets, and fats groups. In light of these findings, we did control for the effects of underreporting in our study.

In the present study, we found a significant and inverse association between milk intake and BMI and %BF only in girls. Another study has also found the same association [38]. Overall, the evidence suggests that gender may influence body composition, with girls having greater %BF [12, 51]. We cannot exclude the hypothesis that the interaction between DPs (and its components) and weight and body fat may differ across different thresholds of %BF [32]. For instance, Vergnaud et al. [56] reported that milk and yogurt intake were protective against 6-year changes in body weight only in adults who were initially overweight. In line with this, dos Santos et al. [15] found a negative relationship between calcium intake and body trunk fat only in obese adolescents. However, in our study, DPs (total, milk, yogurt, and cheese) consumption was not associated with %BF when normal weight and overweight/obese adolescents were considered separately (data not shown). Furthermore, the dynamic metabolic changes that occur during growth and puberty may complicate the interaction between DPs and weight and body fat [21]. Nevertheless, this study addressed sexual maturation, controlling for the extent of biological growth and the individual nutritional needs of adolescents [47]. Although no inverse association was found in boys, consumption of DPs did not increase the probability of being obese, as other studies had reported [2, 23, 36]. It is noteworthy that DPs are widely recognized as good sources of nutrients in the adolescents' diet as they are important to in the promotion of bone and overall health [54].

Several mechanisms were proposed to explain how DP might influence energy balance and body composition. DPs are an important source of calcium, which appears to play a significant role in the regulation of energy metabolism by reduction of lipogenesis and enhancement of lipolysis on adipocyte, increasing both fecal fat excretion and fat oxidation [16]. In the present study, however, we found a significant association only for milk consumption, even after adjusting for calcium, and other plausible mechanisms should be taken into account. Recently, it has been suggested that milk is rich in bioactive peptides (whereas other DPs contain little or no such substances) that may also act independently of calcium to modulate body fat accumulation [40, 45]. Milk bioactive peptides (casokinins and lactokinins) have been shown to inhibit angiotensin-converting enzyme and, consequently, to inhibit the production of angiotensin II hormone, which has been reported to upregulate adipocyte lipogenesis, resulting in the inhibition of fat deposition [61]. In addition, Strazzullo et al. [49] showed, in a prospective study with adult men, that carriers of the DD variant of the angiotensin-converting enzyme gene (associated with higher plasma levels of angiotensin-converting enzyme activity) reported a higher incidence of overweight. Milk, especially whey protein, also stimulates insulin secretion that may directly affect food intake regulation by suppressing appetite, independent of the effects of dietary Ca [27, 35]. Yet, we could not exclude the possibility that milk consumption might be a marker of other healthier lifestyle traits that protect against overweight/obesity and that were not explored in the present study. A positive health relationship between milk intake and education may reflect a growing concern about health in the higher socioeconomic groups. However, the association between milk consumption and socioeconomic position is sometimes contradictory [13, 43]; therefore, our results were adjusted for parental education.

Our study has some limitations. Its cross-sectional design prevents the drawing of any conclusions related to cause and effect. The measures of %BF used in this study are also less accurate than more sophisticated measurements (i.e., DXA, computerized tomography, magnetic resonance imaging). However, BIA has been used as a simple, reliable, valid, inexpensive, portable, and quick tool to assess %BF with large samples, and BIA has also been better correlated than anthropometric indices (BMI and weight-for-height index) in estimations of %BF [53].

In conclusion, we found an inverse association between milk intake and BMI and %BF in girls. Further studies are needed on the roles that diet and healthy lifestyles play in the interaction between DPs and body composition. Our findings may also encourage further research on the effects of a threshold BF and sex hormone effects explaining the difference between girls and boys as well as the influence of additional dairy components, such as whey proteins.

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Conflict of interest The authors declare no conflict of interest.

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Paper IV

[Submitted – *under review*]

Relationship of milk intake and physical activity to abdominal obesity among adolescents

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ABSTRACT

Background: Diet and physical activity (PA) are recognized as important factors to prevent abdominal obesity (AO), which is strongly associated with chronic diseases. Some studies have reported an inverse association between milk consumption and AO.

Objective: This study examined the association between milk intake, PA and AO in adolescents.

Methods: A cross-sectional study was conducted with 1,209 adolescents (503 boys), aged 15-18 from the Azorean Archipelago, Portugal in 2008. Anthropometric measurements were recorded (weight, height, waist circumference-WC). AO was defined by a WC at or above the 90th percentile. Adolescent food intake was measured using a semi-quantitative food frequency questionnaire, and milk intake was categorized as 'low-milk-intake' (<2 servings/day) or 'high-milk-intake' (≥2 servings/day). PA was assessed via a self-report questionnaire, and participants were divided into active (>10 points) and low-active groups (≤10 points). They were then divided into four smaller groups, according to milk intake and PA: (i) low-milk-intake/low-active; (ii) low-milk-intake/active; (iii) high-milk-intake/low-active; (iv) high-milk-intake/active. The association between milk intake, PA and AO was evaluated using logistic regression analysis, and the results were adjusted for demographic, body mass index, pubertal stage and dietary confounders.

Results: The group of adolescents with high level of milk intake and active had a lower proportion of AO than did other groups (low-milk-intake/low-active: 34.2%; low-milk-intake/active: 26.9%; high-milk-intake/low-active: 25.7%; high-milk-intake/active: 21.9%, P=0.008). After adjusting for confounders, low-active and active adolescents with high levels of milk intake were less likely to have AO, compared to low-active adolescents with low milk intake (high-milk-intake/low-active, OR=0.412, 95% CI: 0.201–0.845; high-milk-intake/active adolescents, OR=0.445, 95% CI: 0.235–0.845).

Conclusion: High milk intake seems to have a protective effect on AO, regardless of PA level.

Keywords: Abdominal obesity; adolescents; milk; physical activity.

INTRODUCTION

In recent decades, the prevalence of obesity, including abdominal obesity (AO), has significantly increased among adolescents (1-4). These findings are alarming, in view of the fact that AO is an independent risk factor for insulin resistance, hyperinsulinemia, dyslipidemia and hypertension in youth (5, 6). Despite the upward trend in obesity, there is still a lack of knowledge of the factors associated with AO in adolescents. It has been reported that diet and physical activity (PA) play an important role in the prevention of AO (7, 8). Several observational and prospective studies have suggested that milk or milk product intake is associated with lower risk of excessive adiposity in children, adolescents and adults (9-12). The weight of evidence suggests that milk intake is more likely to be associated with beneficial weight and body fat than other milk products such as yogurt or cheese (9, 13-15). It has been suggested that milk is rich in bioactive peptides (whereas other milk products contain little or no such substances) that may modulate body fat accumulation (16-18). On the other hand, other studies have found a positive (19) or null association (20) of milk intake with adiposity.

The emergence of the prevalence AO and related conditions, parallels with the decreasing levels of PA, and increasing levels of time spent on sedentary activities (television watching, video game playing and computer use) has increased among adolescents (21, 22). As recently noted, the practice of structured and vigorous PA is inversely associated with excess central adiposity (7). Despite the overwhelming evidence of the association between PA and body composition, PA levels are also linked to other healthy-related factors, lifestyle patterns and psychosocial well-being (23).

Although evidences suggest that milk intake and PA have an independent role in AO, gaps remain in the literature on the combined effects. Moreover, these two lifestyle habits are not mutually exclusive and often coexist in the same individual (24). In a recent review it is suggested that more research is needed in order to compare the combined effects of milk products consumption and PA on body composition (25). To the best of our knowledge, no study has examined this combined association in adolescents. In this context, the aim of this study was to identify the association of milk intake and PA on AO in a sample of Portuguese adolescents.

MATERIALS AND METHODS

Sampling

Data for the present cross-sectional study were derived from a school-based study – The Azorean Physical Activity and Health Study II, which aimed to evaluate PA, physical fitness, overweight/obesity prevalence, dietary intake, health-related quality of life and other factors in 15 to 18 years old adolescents, in 2008. This study was carried out in 6 of the 9 Azorean Islands (S. Miguel, Terceira, Faial, Pico, S. Jorge and Graciosa), where 95% of the Azorean population lives (26).

All participants in this study were informed of its goals, and the parent or guardian of each participant provided written informed consent for his/her child to participate. The study was approved by the Faculty of Sport, University of Porto and the Portuguese Foundation for Science and Technology Ethics Committee; it was conducted in accordance with the World Medical Association's Helsinki Declaration for Human Studies.

The population was selected by means of proportionate stratified random sampling, taking into account location (island) and number of students, by age and sex, in each school. The estimated number of subjects for the representativeness of adolescent population was 1,422, but in order to prevent incomplete information, data was collected for 1,515 adolescents. Some adolescents were not included in our analysis (n=306), because information was missing on their dietary intake (n=286) and waist circumference (WC) (n=20). This resulted in the collection of data for a total of 1,209 participants (503 boys). The subjects who were excluded from this study did not significantly differ from those who were included, with regard to age (16.2±1.0y vs. 16.1±1.0y, P=0.158), parental education (9.1±4.5y vs. 9.1±4.4y, P=0.890) and gender (girls: 61.1% vs. 58.4% and boys: 38.9% vs. 41.6%, P=0.388). Finally, the sample was weighted in accordance with the distribution of the Azorean population in schools and so as to guarantee the real representativeness of each group (by age and gender).

Anthropometric measures

Body Height and Body Weight

Body height and body weight were determined using standard anthropometric methods. Height was measured to the nearest mm in bare or stocking feet, with adolescents standing upright against a Holtain portable stadiometer (Crymych, Pembrokeshire, UK). Weight was measured to the nearest 0.10 kg, with participants lightly dressed (underwear and t-shirt) and with the use of a portable digital beam scale (Tanita Inner Scan BC 532, Tokyo, Japan).

Body mass index (BMI) was calculated using the ratio of weight/height2 (kg/m2). Subjects were classified as normal weight, overweight or obese, according to age- and sex-specific cut-off points specified by the International Obesity Task Force (27, 28). Underweight subjects (2.6%) were combined with subjects in the normal weight category, due to the fact that they represented a small proportion of the sample. Percentage of body fat (%BF) was assessed using bioelectric impedance analysis (Tanita Inner Scan BC 532, Tokyo, Japan).

Waist Circumference

WC measurements were taken midway between the tenth rib and the iliac crest and recorded to 0.1 cm. A non-elastic flexible tape measure was used, with subjects standing erect – arms by sides, feet together and abdomen relaxed – as well as without clothing covering the waist area. Subjects were divided into two categories (<90th and ≥90th percentiles), according to age- and sex-specific cut-off points specified by Sardinha et al. (29). Subjects who were in the 90th percentile or above were considered to have AO (30).

Pubertal stage

To determine pubertal stage (which ranged from 1 to 5), each subject was asked to self-assess his/her stage of development of secondary sex characteristics. Breast development in girls and genital development in boys was evaluated according to criteria outlined by Tanner and Whitehouse (31). Adolescents in Tanner stage 1 (0.4%) were combined with subjects in the Tanner stage 2, due to the fact that they represented a small proportion of the sample.

Socio-demographic and lifestyle variables

Participants answered a questionnaire that assessed several socio-demographic and lifestyle variables.

Smoking

Participants were classified as non-smokers, former smokers (individuals who had stopped smoking for at least six months), occasional smokers (individuals who smoked, on average, less than one cigarette a day) and current smokers (individuals who smoked at least one cigarette a day) (32). Occasional smokers were recoded and combined with current smokers, due to the fact that they represented a small proportion of the sample.

Parental Education

For the present study, highest level of parental education (measured by number of school years completed) was used as a proxy measure of socio-economic status. Participants were divided into three categories, reflecting divisions within the Portuguese educational system: mandatory or less (≤ 9 school years), secondary (10 to 12 school years) and college/university (> 12 school years).

Dietary intake

Dietary intake was measured via a self-administered semi-quantitative food frequency questionnaire (FFQ), validated for the Portuguese adults (33). This semi-quantitative FFQ was designed in accordance with criteria laid out by Willett et al. (34) and adapted to include a variety of typical Portuguese food items. The FFQ was adapted for adolescents by including foods more frequently eaten by this age group (35); the adolescent version covered the previous 12 months and comprised ninety-one food items or beverage categories. For each item, the questionnaire offered nine frequency response options, ranging from 'never' to 'six or more times per day', and standard portion size and seasonality. Any foods not listed in the questionnaire could be listed by participants in a free-response section. Energy and nutritional intake were estimated with regard to respondents' ratings of the frequency, portion and seasonality of each item, using the software Food Processor Plus (ESHA Research Inc., Salem, OR, US). This program uses nutritional information from the United States that has been adapted for use with typical Portuguese foods and beverages.

The amount of milk (whole, semi skimmed and skimmed) that counted as a single serving was considered to be 250 ml. We included all types of milk in one variable because the majority of adolescents were consuming semi skimmed (83.7%) or skimmed milk (8.6%). Participants were categorized according to the new Portuguese Food Wheel guide (36) and adolescents who consumed two or more servings of milk per day were included in the ‘high-milk-intake group’, while those who consumed less than two servings per day comprised the ‘low-milk-intake group’.

Physical activity

Physical activity was assessed via a self-report questionnaire that evaluated leisure-time physical activities (37). This questionnaire has been shown to have good test-retest reliability among Portuguese adolescents (intraclass correlation coefficient: 0.92–0.96) (38). It consists of five questions with four answer choices (each rated on a four-point scale): (1) Outside school, do you take part in organized sports/physical activities?; (2) Outside school, do you take part in non-organized sports/physical activities?; (3) Outside school hours, how many times a week do you take part in sports or physical activities for at least 20 minutes?; (4) Outside school hours, how many hours a week do you usually take part in physical activities, so much that you get out of breath or sweat?; (5) Do you take part in competitive sports?. The maximum number of points possible was 20. A PA index (PAI) was obtained for each respondent by totaling his/her points, which corresponded to activity level rankings that ranged from ‘sedentary’ to ‘vigorous’. Participants whose PAIs were greater than 10 points were classified as ‘active’, while those whose physical activity indices were 10 points or less were classified as ‘low-active’ (38).

Statistical analysis

For the purposes of this study, participants were divided into four groups, according to their milk intake (high or low) and PA (active or low-active): (i) low-milk- intake/low-active; (ii) low-milk-intake/active; (iii) high-milk-intake/low-active; and (iv) high-milk-intake/active.

The Kolmogorov–Smirnov test was used to assess the assumption of normality. One-way analysis of variance (ANOVA), with the Bonferroni post-hoc test, was performed to compare continuous variables, and the Chi-square test was used to test for categorical variables across groups. When the continuous variables were found to not be normally distributed, the Kruskal-Wallis test was used to determine differences between groups, and the Mann-Whitney test was used to examine unique pairs. In this report, descriptive analysis is presented in terms of means and standard deviations, unless otherwise stated.

A multivariate logistic regression model was constructed to verify the relationship between AO and the combined associations of milk intake and PA, adjusting for age (in years), gender (reference – boys), parental education (reference – mandatory or less), BMI (reference – obese), pubertal stage, energy intake (in kcal), total calcium intake (in mg), protein intake (g/kg), total fat intake (% of energy) and dietary fiber (in g/1000 kcal). Age and dietary variables were entered as continuous variables. Furthermore, we adjusted the logistical model by underreporting energy intake, which was estimated using the ratio between reported energy intake and predicted basal metabolic rate (EI:BMR) (39-41). The thresholds that defined low-energy reporters (underreporters) were 1.70 and 1.71 for girls and boys between 15 and 17 years old and 1.67 and 1.81 for girls and boys age 18. 'Low-energy reporter' (a categorical variable) was included in the model as a confounding factor.

Odds ratios (OR) and 95% confidence intervals (CI) were computed across groups, with the 'low-milk-intake/low-active' group as the reference group. A P-value of <0.05 was regarded as significant. All analyses were performed using PASW Statistic v.18 (SPSS, Chicago, Illinois, US).

RESULTS

Descriptive characteristics of the adolescents in the sample are shown in Table 1. The higher proportion of girls was seen in the low-activity groups, regardless milk intake (P<0.001). Adolescents with high milk intake and active had higher body weight compared to adolescents with high milk intake and low-active (61.4 (14.4) vs. 58.6 (14.6), P=0.001, respectively); and higher body height and lower %BF compared to compared to adolescents with high milk intake and low-active and with low-milk intake and low-active (P<0.001, for all).

Active adolescents had lower %BF compared to low-active adolescents, regardless milk intake (*P*<0.001, for all). The higher proportion of parents with mandatory or less education was seen in 'low-milk-intake/low-active' group (*P*<0.001). No significant differences were seen in age, BMI, pubertal stage and smoking across groups.

Table 1 - Characteristics of the study sample, by milk intake and physical activity groups.

	Total (n=1209)	Milk intake, physical activity level: Low milk, low-active (n=260)	Low milk, active (n=417)	High milk, low-active (n=167)	High milk, active (n=365)	*P*
Age[c, d] (years)	16.0 (2.0)	16.0 (2.0)	16.0 (2.0)	16.0 (2.0)	16.0 (2.0)	0.215
Weight[c, d] (kg)	60.5 (15.0)	58.5 (13.6)	62.2 (15.2)*	58.6 (14.6)†	61.4 (14.4)*†§	<0.001
Height [c, d] (m)	1.65 (0.12)	1.61 (0.12)	1.66 (0.14)*	1.63 (0.10)†	1.67 (0.14)*†§	<0.001
BMI[c, d] (kg/m^2)	22.1 (4.3)	22.3 (4.6)	22.1 (4.2)	21.6 (4.8)	21.9 (4.0)	0.160
Weight status (%)[e]						
Normal	69.2	66.1	68.8	68.8	71.8	
Overweight	23.2	25.8	23.3	23.4	21.4	0.881
Obese	7.6	8.1	7.9	7.8	6.8	
Body fat[a, b] (%)	21.6 (8.8)	25.0 (8.0)	20.3 (9.1)*	23.9 (8.9)†	19.6 (8.3)*§	<0.001
Waist circumference[c, d] (cm)	78.0 (13.0)	80.0 (14.0)	78.0 (13.0)	77.0 (14.0)*	76.0 (12.0)*†	0.017
Gender [e] (%)						
girls	58.4	83.1	47.0	80.2	43.8	<0.001
boys	41.6	16.9	53.0	19.8	56.2	
Pubertal stage[e] (%)						
Tanner stage 1 or 2	1.0	1.5	0.5	1.2	1.1	
Tanner stage 3	15.7	16.2	13.2	23.4	14.8	
Tanner stage 4	59.1	58.1	61.9	51.5	60.0	0.191
Tanner stage 5	24.2	24.2	24.5	23.9	24.1	
Parental education [e] (%)						
mandatory or less	48.6	61.7	47.1	47.6	41.2	
secondary	36.5	32.3	37.1	39.2	37.6	<0.001
college /university	14.9	6.0	15.8	13.2	21.2	
Smoking status[e] (%)						
Non smoker	87.0	89.6	86.6	86.2	86.0	
Former smoker	5.7	3.5	6.5	4.2	7.1	0.384
Occasional/Current smoker	7.3	6.9	6.9	9.6	6.9	
PAI[c, d]	13 (8)	8 (3)	15 (5)*	8 (3)†	16 (5)* §	<0.001

BMI - body mass index; PAI – physical activity index; [a] Data are mean (standard deviation); [b] analysis by ANOVA for continuous variables; [c] Data are median (interquartile range); [d] analysis by Kruskal-Wallis for continuous variables; [e] analysis by χ^2 for categorical variables. * *P*<0.05, compared to the low-milk-intake/low-active group; † *P*<0.05, compared to the low-milk-intake/active group; § *P*<0.05, compared to the high-milk-intake/low-active group.

Adolescents with high milk intakes had lower proportions of AO, compared with those who had low milk intakes (23.1% vs. 29.7%, *P*=0.006, respectively). Active adolescents also had lower proportions of AO than low-active adolescents (24.6% vs. 30.9%, *P*=0.011, respectively).

The energy intakes and dietary characteristics of each group are presented in Table 2. Regardless of whether they were active or low-active, adolescents whose milk intakes were high had higher levels of energy and total calcium and protein intake, compared with those who had low milk intake (*P*<0.05). There was no significant difference across groups with regard to carbohydrates and total fat intake.

Table 2 - Dietary characteristics of the study sample, by milk intake and physical activity groups.

	Total	Milk intake, physical activity level				
		Low milk, low-active (n=260)	Low milk, active (n=417)	High milk, low-active (n=167)	High milk, active (n=365)	*P*
	(n=1209)					
Energy intake[c, d] (kcal/day)	2301.2 (1431.1)	2070.9 (1343.3)	2044.3 (1458.3)	2544.3 (1358.4)*†	2621.9 (1513.4)*†	<0.001
Protein[a, b] (% of energy)	17.8 (3.8)	16.7 (3.5)	17.7 (4.2)*	18.3 (3.6)*	18.4 (3.8)*	<0.001
Protein[c, d] (g/ kg body weight)	1.6 (1.0)	1.4 (1.0)	1.4 (1.0)	2.0 (1.0)*†	1.9 (1.2)*†	<0.001
Carbohydrate[a, b] (% of energy)	49.3 (7.9)	50.4 (8.3)	48.8 (8.4)	49.2 (7.4)	49.1 (7.1)	0.082
Total fat[a, b] (% of energy)	32.3 (5.7)	32.4 (5.7)	32.7 (5.7)	32.1 (5.4)	31.9 (5.3)	0.329
Dietary fiber[c, d] (g/1000kcal)	9.4 (4.2)	9.9 (4.2)	9.6 (4.6)	9.1 (3.8)†	9.1 (3.8)*†	0.004
Total calcium intake[c, d] (mg/day)	1120.6 (419.0)	767.7 (230.3)	818.5 (251.6)	1579.9 (434.0)*†	1560.1 (456.8)*†	<0.001
Milk intake[c, d] (servings/day)	1.0 (0.8)	0.9 (0.3)	1.0 (0.3)	2.4 (0.1)*†	2.4 (0.1)*†	<0.001

[a] Data are mean (standard deviation); [b] analysis by ANOVA for continuous variables; [c] Data are median (interquartile range); [d] analysis by Kruskal-Wallis for continuous variables; * *P*<0.05, compared to the low-milk-intake/low-active group; † *P*<0.05, compared to the low-milk-intake/active group.

The proportion of AO across milk intake and PA groups, as indicated in Figure 1, showed that adolescents with high levels of milk intake and activity had lower proportions of AO, compared to other groups (low-milk-intake/low-active: 34.2%, vs. low-milk-intake/active: 26.9%, vs. high-milk-intake/low-active: 25.7%, vs. high-milk-intake/active: 21.9%, *P*=0.008).

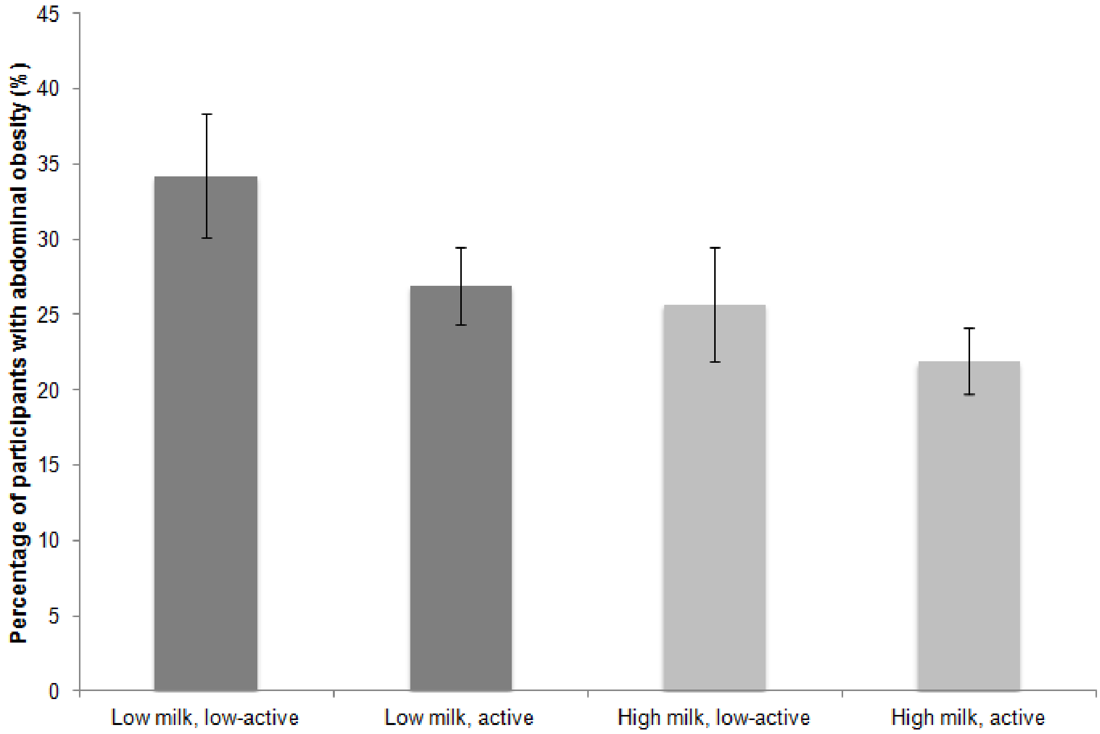

Figure 1 - Proportion of subjects with abdominal obesity across milk intake and physical activity groups.

The results of the multivariate logistic regression analysis, predicting AO from a combination of milk intake and PA, are shown in Table 3. After adjusting for demographic and dietary variables, low-active and active adolescents with high milk intakes were less likely to have AO than were low-active adolescents with low milk intakes (low-milk-intake/low-active, OR=0.928, 95% CI: 0.562–1.531; high-milk-intake/low-active, OR=0.412, 95% CI: 0.201–0.845; high-milk-intake/active, OR=0.445, 95% CI: 0.235–0.845). To demonstrate whether it is really milk intake making the difference we made an additional multivariate logistic regression analysis with 'low-milk-intake/active' group as the reference group. Low-active and active adolescents with high levels of milk intake remain to be less likely to have AO even after compared to active adolescents with low milk intake (low-milk-intake/low-active, OR=1.078, 95% CI: 0.653–1.779; high-milk-intake/low-active, OR=0.444, 95% CI: 0.217–0.909; high-milk-intake/active adolescents, OR=0.480, 95% CI: 0.263–0.876).

Table 3 - Odds ratio for abdominal obesity by milk intake and physical activity groups.

		Model 1			Model 2		
		OR	**95% CI**	***P*[a]**	**OR**	**95% CI**	***P*[a]**
Low milk	Low-active	1	reference	0.008	1	reference	0.027
	Active	0.706	0.501-0.987		0.928	0.562-1.531	
High milk	Low-active	0.666	0.433-1.025		0.412	0.201-0.845	
	Active	0.539	0.378-0.770		0.445	0.235-0.845	

OR – odds ratio; CI – confidence interval; 1 – reference category.
[a] *P-value* for heterogeneity.
Model 1 – unadjusted model.
Model 2 – adjusted for age (years), gender (reference – boys), parental education (reference – mandatory or less), body mass index (reference - obese), pubertal stage, low-energy reporter, energy intake (kcal), total fat intake (% of energy), total calcium intake (mg), protein intake (g/kg body weight) and density fiber (g/1000kcal).

DISCUSSION

The present study explored the combined association of milk intake and PA on AO in adolescents. The results suggested that adolescents with high milk intakes, regardless of whether they were active or low-active, were less likely to have AO, compared to those who had low milk intakes. This association was not confounded by other lifestyle factors or nutritional variables, as it remained significant after adjustments.

Most cross-sectional and prospective studies have found an inverse relationship between milk intake or milk products and BMI, body weight and/or body fat in children and adolescents (9, 14, 42, 43). Furthermore, evidence shows that the consumption of milk and milk products does not affect negatively weight and body composition (44). However, studies examining the association between milk intake or milk products and AO are limited. In one study with children, increased milk consumption was associated with lower WC (45). Bradlee and colleagues (46) analyzed data from NHANES III and also found that mean dairy intake was inversely associated with central obesity in adolescents. Previous results, derived from the same sample, had shown that dairy product intake had a protective association with AO in boys (47). A randomized controlled trial on the effects of a dairy-rich diet on AO with obese children showed that those with isocaloric dairy-rich diets (> 800 mg calcium/day) had lower WC than other groups (i.e. controls with and without energy restrictions) at the end of the study (48). It is noteworthy that, in addition to its possible ‘antiobesity’ effect, milk is an excellent source of nutrients (such as calcium) for adolescents, who experience a period characterized by dynamic changes that occur in response to growth and puberty. Hence, consumption of recommended amounts of milk may help adolescents meet their nutrient requirements and improve their diets’ quality (44, 49). Moreover, moderate evidence shows that intake of milk and milks products is linked to improved bone health in children and adolescents and is also associated with reduced risk of cardiovascular disease, type 2 diabetes and blood pressure in adults (50).

In this study, we also found that active adolescents had lower proportion of AO. PA also protects against central adiposity by increasing immediate energy requirements, which causes important changes in fuel utilization and mobilization (51). Evidence suggests that low levels of leisure-time PA are associated with AO in youth (7). Klein-Platat et al. (52) reported that, in an adolescent sample, AO was negatively associated with structured PA (outside school, > 140 min/week) and positively associated with sedentary activities (e.g., television, watching). By the same token, Ortega et al. (53) found that children and adolescents in the lowest tertile of vigorous PA had higher odds of having high WC, when compared with those in the highest tertile.

Concerning dietary habits and PA levels, some studies have found that the consumption of food by more active and less active adolescents differs (24, 54, 55). In a study by Ottevaere et al. (24), the most active adolescents consumed more milk products than did their less active counterparts. However, previous research indicates that spending more time on PA does not exclusively result in healthier eating habits (24, 55). Although, in our study, the majority of active adolescents showed low milk intake, active adolescents with high milk intake, when compared to other groups, had lower proportions of AO, which shows the relevance of exploring both dietary patterns and PA simultaneously when assessing AO in adolescents.

When interpreting the results of the combined association between milk intake and PA, we observed that adolescents with high milk intake showed lower odds of expressing AO, regardless of their PA levels. This results is further enhanced by the lack of significance between groups with low milk intake where there was no significant differences between be active or low-active. Thus, this finding suggests that the consumption of milk may overcome the potentially negative effects of low PA levels on the likelihood of having abdominal obesity. Furthermore, milk compounds may also be involved in body fat distribution. Visceral adipose tissue has greater amounts of 11-β-hidroxysteroid dehydrogenase type 1 (56), which is over-expressed in-vitro in those with central adiposity (57). It has been suggested that a high-calcium and high-dairy diet down-regulates 11-β-hidroxysteroid dehydrogenase type 1 expression and decreases the concentration of glucocorticoid, which consequently decreases the size of adipose fat deposits (56). However, we cannot exclude the hypothesis that milk consumption may also be associated with other healthy eating habits and healthier lifestyles, which may protect against AO.

We also found that although energy intake is higher in adolescents with high milk consumption the additional energy intake does not result in higher odds of AO. In a study to determine the effects of a calcium-rich diet on weight gain during 2 years, girls 9 year old were randomly assigned to a supplying at least 1,500 mg (primarily from dairy food) of calcium per day or their usual diet (58). Although girls in the calcium-rich diet group consumed approximately 150 more calories per day, they did not have greater increases in body weight, BMI, or fat or lean mass compared to the usual diet group. Furthermore, girls who consumed calcium-rich diet also significantly increased their intake of essential nutrients including calcium, protein, vitamins A and D, phosphorus and magnesium compared with girls on their usual diets. In adults, Zemel et al (59) conducted a 9 months randomized trials to compare the effects of low- (< 1 serving/day) and high-dairy diet (> 3 serving/day) on weight maintenance. Although the results showed that the high dairy diet group had a higher energy intake than the low dairy group, there were no differences in weight and body composition between the two groups after the intervention. Thus, the author suggested that the high dairy diet group exhibited evidence of greater fat oxidation and was able to consume greater energy without greater weight gain compared to the low dairy group.

Some limitations to our study should be addressed. First, it should be noted that, as in other cross-sectional studies, conclusions related to cause and effect cannot be drawn. Second, the measure of AO used in this study is an indirect estimate of abdominal fat and there are some sophisticated methods to accurately measure abdominal fat, such as magnetic resonance imagining or dual-energy X-ray absorptiometry. However, such techniques are not feasible to apply in large epidemiological studies because they are complex, time consuming and expensive. Furthermore, it has been suggested that WC is one of the most common proxy measures of AO (1) and is strongly associated with visceral adipose tissue (60). Finally, with the use of self-reported PA and dietary intake data, one cannot rule out some reporting bias. Yet both questionnaires have been previously tested (33, 38), and analysis was controlled to prevent the misreporting of energy intake.

In conclusion, we found that high milk intake seems to have a protective effect on AO, regardless of PA levels in adolescents. Future prospective and randomized clinical investigations, with more accurate measures, are needed on the combined effects of milk intake and/or milk products and PA on AO.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Chapter 3

Overall discussion

The main findings of the studies presented in this dissertation suggested an inverse association between some foods, particularly dairy products, and obesity in adolescents. Moreover, our results suggested that milk intake is more likely to be associated with beneficial BMI, body fat and WC outcomes than other dairy products, such as yogurt and cheese. Data from cross-sectional (Barba et al., 2005; Black et al., 2002; Bradlee et al., 2010; Fiorito et al., 2006; Forshee & Storey, 2003; Hirschler et al., 2009; LaRowe et al., 2007; Moore et al., 2008; Murphy et al., 2008; Olivares et al., 2004; Rockett et al., 2001; Roseman et al., 2007; Tanasescu et al., 2000) and prospective (Carruth & Skinner, 2001; Johnson et al., 2007; Moore et al., 2006; Rockell et al., 2005) epidemiological studies also support the hypothesis that a dairy food-rich diet is associated with lower BMI, body fat and WC in children and adolescents. Although few studies have examined the roles of different dairy products, there is evidence that 'lower'-fat dairy products, such as milk, are associated with lower adiposity (Dougkas et al., 2011). Thus, our results are in line with this evidence and supported by it.

It is believed that the beneficial role of milk is due to the presence of biologically-active components that affect adipocyte metabolism, fat oxidation and absorption and the regulation of food intake and appetite (Dougkas et al., 2011). However, these physiological pathways have not been examined in children and adolescents (Huang & McCrory, 2005). Adolescence is characterized by significant somatic growth and the maturation of secondary sexual characteristics (Lobstein et al., 2004). Somatic growth is coordinated primarily through the action of growth hormone (GH), which is the principal regulator of the hepatic synthesis of insulin-like growth factors (IGF). The IGF family consists of insulin, IGF-I and IGF-II. IGF-I is a polypeptide that exerts anabolic effects on amino acid and carbohydrate metabolism, increases muscle mass and is a major regulator of bone growth during childhood and adolescence (Bonefeld & Moller, 2011; Hill et al., 2008). Serum IGF-I increases during puberty, with the highest concentrations seen in Tanner stage 3-4 in girls and Tanner stage 4 in boys (Hoppe et al., 2006). Concentration of IGF-I appears to be influenced by several factors, including age, sleep, physical activity, body fat and nutrition - and particularly by energy, protein and certain micronutrient intake (Hoppe et al., 2006). There is some evidence that milk consumption can stimulate the circulation of IGF-I and influence growth (Hoppe et al., 2006). Furthermore, IGF-I seems to be a strong predictor of calcium retention in adolescent boys (Hill et al., 2008). However, it appears that nutritional regulation of IGF-I is more important during infancy (when IGF-I concentration is low) than in later adolescence. Thus, the possible role of milk intake in stimulating IGF-I during adolescence is unclear.

In addition to its possible beneficial effects on obesity, milk is a complete source of nutrients, comprising all of the major macronutrients and some micronutrients, particularly calcium (Agostoni & Turck, 2011). Several studies have demonstrated that when milk is largely excluded from adolescents' diets, it is difficult to achieve daily requirements for calcium and other nutrients (Albertson et al., 1997; Gao et al., 2006; Goolsby et al., 2006; Weaver, 2010). The new Portuguese Food Wheel guide acknowledges the important dietary role of dairy products and recommends that the Portuguese population consume at least 2 servings per day of milk or milk equivalents (i.e., yogurt or cheese) as part of a healthful diet (Rodrigues et al., 2006). Two servings of milk per day (i.e., 500 ml/day) provide 614 mg of calcium (Instituto Nacional de Saúde Dr. Ricardo Jorge, 2007), which correspond to 47% of the recommended calcium intake for adolescents by the Institute of Medicine (Ross et al., 2011). Interestingly, it has been suggested that there is a possible threshold effect of 600-800 mg of dietary calcium intake, above which fat loss is enhanced. A recent review of a mixed-model regression analysis, based on 18 trials in adults, showed that an increase in calcium intake from 400 to 1200 mg per day would be associated with a decrease in BMI, from 25.6 to 24.7 kg/m^2 (Dougkas et al., 2011).

It has also been shown that milk and dairy product consumption may be a marker of overall healthy diet (Spence et al., 2011). For example, it is widely established that RTECs, which are an important source of several nutrients, are most frequently consumed with milk (Kafatos et al., 2005; Kosti et al., 2010). The beneficial association found in one of our studies (Paper I) between RTEC intake and abdominal obesity leads us to the hypothesis that this result may be partly due to the effects of milk intake. Yet, other explanations for the relationship between RTEC and obesity might be considered. RTEC consumption may be a marker for other healthful lifestyle factors, such as breakfast consumption (Kosti et al., 2010), high levels of physical activity and reduced television viewing (Albertson, Affenito, et al., 2009; Albertson et al., 2008). Furthermore, several varieties of RTEC are important food sources of dietary fiber, which has been found to be inversely associated with obesity (Davis et al., 2009; van de Vijver et al., 2009).

In many observational studies, it remains possible that milk and dairy consumption is a marker of SES, with lower intake seen in lower SES households (Deshmukh-Taskar et al., 2007; Larson et al., 2006; Neumark-Sztainer et al., 1997). It has been postulated that the more healthful food consumption seen in higher SES households may be due to the knowledge and health awareness or the increased pressures to attain social acceptability that occur with increasing SES (Deshmukh-Taskar et al., 2007). Parental awareness of health may help children and adolescents develop strategies for choosing healthy foods at home and in social situations. In the present dissertation, although we did not explore the relationship between SES and dairy consumption, we cannot exclude the hypothesis that these two factors may be related. In addition, the inverse association between father's employment status and abdominal obesity found in one of our studies (Paper I) is in accordance with a recent review that proposed that father's employment is a probable early marker of obesity (Brisbois et al., 2012).

The findings of this thesis also reveal the need to disaggregate dairy products in analyses of obesity outcomes. Dairy processing generates a variety of biochemical changes to milk's composition, including the loss of more labile constituents (e.g., vitamin C, enzymes), the removal of bioactive components (e.g., whey removal from cheese) and/or the addition of ingredients (e.g., the addition of sugar to yogurt and salt to cheese) (Wiley, 2010). Thus, the use of total dairy intake may not reveal the underlying relationships between milk and obesity parameters in adolescents (Wiley, 2010).

In this dissertation, we also explored the relationship between physical activity and measures of obesity. Although, in our regression analysis, no association was seen between physical activity and obesity, we should highlight the fact that adolescents with abdominal obesity were less active than their leaner counterparts. These findings are in accordance with some evidence that physical activity has a beneficial impact on abdominal obesity (Klein-Platat et al., 2005; YoonMyung & SoJung, 2009). In addition, we also found that adolescents with higher milk intake and activity levels were taller and heavier but had lower %BF than adolescents who were less active and had higher high milk intake. It is well established that increased physical activity is associated with lower fatness (Must & Tybor, 2005), and thus the combined effect of physical activity and milk intake may enhance beneficial obesity outcomes.

The limitations of this dissertation were described in detail in all four papers.

As obesity becomes a major public health problem worldwide, there is an urgent need to identify modifiable dietary and lifestyle risk factors for obesity. Thus, intake of dairy products, particularly milk, an easily accessible and nutrient-rich food, as well as the practice of physical activity, should be considered in the prevention and treatment of childhood obesity.

Chapter 4

Conclusions

Based on the purpose and findings of the present dissertation, we emphasize the following conclusions:

1. Higher milk and ready-to-eat cereal intake, as well as father's employment status, have protective effects on abdominal obesity;
2. Physical activity seems to be lower in adolescents with abdominal obesity;
3. Milk intake is more likely to be associated with beneficial BMI, body fat and WC outcomes than is the intake of other dairy products, such as yogurt and cheese;
4. High milk intake seems to have a protective effect on abdominal obesity, regardless of physical activity level.

Perspectives for future research

The results of this dissertation emphasize the need for more research on the relationship between dairy product intake and obesity, that is: for investigation into the mechanisms underlying the 'anti-obesity' effect of dairy intake in adolescents, for randomized controlled studies on the combined effects of dairy product intake and physical activity on obesity and for well-designed studies with more accurate measures of visceral adiposity and with the purpose of examining the effects of different types of dairy products.

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