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The definitive version is available at: La versione definitiva è disponibile alla URL: [http://journals.lww.com/jpgn/pages/articleviewer.aspx?year=9000&issue=00000& article=98274&type=abstract] Impact of snacking pattern on overweight and obesity risk in a cohort of 11-13-y adolescents. Simona Bo, MD, (1), Luca De Carli, MD, (1), Elena Venco, MD, (1), Ilaria Fanzola (1), Maria Maiandi (1), Franco De Michieli (1), Marilena Durazzo, MD, (1), Guglielmo Beccuti, MD, (1), Paolo Cavallo-Perin, MD, (1), Ezio Ghigo, MD, (1), Gian Pasquale Ganzit, MD (2) ¹Department of Medical Science, University of Torino, Torino, Italy ²Sport Medicine Institute of Torino, Italy

Running title: snacking pattern and overweight/obesity

Corresponding author: Simona Bo, Department of Medical Science, University of Torino, Corso Dogliotti 14, 10126 Turin, Italy Telephone +(39)(011)6967864 Fax+(39)(011)6634751 E-mail: simona.bo@unito.it

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Abstract

Objectives: The association between snacking habits and overweight is unclear in adolescents. We evaluated the relationship between snacking patterns and overweight/obesity in a cohort of 11-13-year adolescents. *Methods:* The dietary habits of 400 randomly selected adolescents were evaluated; those with BMI \ge 85th percentile were considered as overweight/obese. Participants were classified by percent caloric intake from snacks (<15%, 15-20, >20%), snacking frequency (1, 2, \ge 3), and timing of consuming the most caloric snack (morning, afternoon, evening). *Results:* A minority (13/400; 3.3%) did not consume any snacks; 5/13 (38.5) of them were overweight/obese. Among snackers (387/400), overweight/obesity prevalences were 10.4%, 14.4%, 20.5% respectively in those consuming <15%, 15-10%, and >20% of their energy intake from snacks. In a Poisson regression model, the overweight/obesity RRs were 1.35 (95%CI 0.58-3.15) and 2.32 (1.10-4.89) for 15-20% and >20% calories/day from snacks, respectively. Overweight/obesity prevalence (from 9.6 to 22.6%) was correlated with snacking frequency (RR=2.20; 0.92-5.27, and RR=4.17; 1.60-10.9, for 2 and \ge 3 snacks/day, respectively). The most caloric snacks were consumed in the morning (180/387) and afternoon (179/387); 28.6% of the predominantly evening snackers (28/387) were overweight/obese (RR = 3.12; 1.17-8.34).

Conclusions: Increased snacking calories, frequency and evening snacking are independently associated with overweight/obesity in Italian middle-school adolescents.

Key words: adolescents, exercise, overweight, snacks

Introduction

Snacking has become a popular habit among children and teenagers [1]. At the same time, overweight and obesity have reached huge proportions, affecting young individuals, particularly in Italy, where the childhood obesity is higher than in most European countries [2]. Snacking has been often considered one of the main contributors to overweight because of the increased consumption of energy-dense, high-sugar, high-fat foods [3-8]. However, other studies failed to establish a correlation between snacking and overweight [9-14]. Such variability could be explained by the definition variability of "snack" [13]. The term "snack" generally designates a small number of items characterized by low nutrient and high energy density (candies, cookies, salty snacks and soda, etc). An alternative definition is based on the time criterion, leading to consider snacks all the eating occasions between main meals [13]. From a nutritional perspective, varying snacks composition with healthier food variety has proved to be a successful strategy in overweight children trying to lose weight [15-16]. According to Drummond, snacking per se may not necessarily predispose to overweight, since a higher eating frequency may be favorable in terms of body weight control and energy balance, lowering caloric intake when replacing main meals [17]. In relation to meal frequency, some authors recognize an inverse relationship between the number of eating occasions and the prevalence of overweight [11,14,18-19]. Finally, the time of snacking might differently impact on metabolism and energy expenditure, as diet-induced thermogenesis is reduced when a test snack is consumed in the evening compared with both morning and afternoon [20], and nighttime snacking decreases whole body fat oxidation [21]. This seems to be valuable, considering the modern trend of eating less in the morning and more over the day in children and adolescents [22].

Even if under-reporting in food questionnaires could affect many of these findings, the debate is certainly still open, and snacking deserves more research because it could help in developing strategies to control or reduce body weight in pediatric subjects. The purpose of this study is to evaluate the relationship between the energy content, frequency, and timing of snacking and overweight/obesity in 11-13-year old adolescents

Subjects and Methods

Participants

All the students attending the first year of middle school in Torino (Italy) routinely undergo a health status and physical performance assessment by the Sports Medicine Institute ("Bambini a Torino" project). In the academic year 2011-2012, 7263 students attended the first year of middle school in Torino. Among them, 6876 participated in the assessment with participation rate of 94.7% (6876/7263).

A week before the assessment, students were administered questionnaires on health status, exercise levels, present and past illness, and medications by the school teachers and were asked to have them filled out by their parents at home. In 400 students, selected by a simple random sampling, a dietary questionnaire was added to the health status questionnaire. The present study was based on these 400 students.

Children's parents or legal representatives signed a consent form for the study participation. The study protocol was approved by the local Ethical Committee, and the procedures were in compliance with the Helsinki Declaration principles.

Measurements

Weight and height were measured in all the participants. Weight was measured to the nearest 0.1 kg, and height was measured to the nearest 0.1 cm with a stadiometer (SECA model 711, Hamburg, Germany), with the participants wearing light clothes and no shoes. Body mass index (BMI) was defined as body mass divided by height square (kg/m^2) , and the BMI percentile values were obtained from the Italian growth charts, referring to Northern/Central Italy percentiles [23]. Subjects who had a BMI \geq 85th percentile were classified as overweight/obese. Data relative to the frequency, duration of each session, and intensity of exercise performed by the participants outside school were collected by the questionnaires administered to the parents.

Exercise was considered as any planned, structured, and repetitive physical activity performed outside gymnastics classes performed at school. The mean weekly exercise level was calculated as the product of duration and frequency of each activity (in hours/week), weighted by an estimate of the metabolic equivalent of the activity (MET), and summed for the activities performed [24]. The whole cohort was divided into tertiles of exercise, and individuals were classified as sedentary, moderately active, and active. The cut-off points in the whole cohort were respectively \leq 10, 11-29, and \geq 30 METs h/week, and were similar to those found in another European cohort of adolescents [25].

Food questionnaires

The parents of the 400 randomly selected subjects were asked to complete both a validated 24-hour food recall questionnaire [26] and a 19-item food-frequency grid with an instruction sheet. In the food-recall questionnaire, different serving sizes were specified for each item, using commonly used portions; furthermore the use of measuring guides (cups, spoons, glasses) aided respondents in estimating the amounts of foods consumed. The temporal pattern of food consumption was recorded, as parents were asked to list foods eaten at each meal, including snacks. The grid evaluated the mean weekly frequency of consumption of 19 items (such as biscuits, fruit juices, soft drinks, fruits, savory snacks, pizzas, crackers, potato chips, popcorn, candies, bread, vegetables, cold cut, sweets, milk/yoghurt, etc) and was developed by using a previously validated semi-quantitative food-frequency questionnaire [27]. The parents indicated how many times a week their child consumed each food included in the list by ticking one of three boxes referring to different ranges of consumption frequency, which varied in accordance to the kind of food. The data obtained from the grid were separately analyzed and were used to check the data collected by the food-recall questionnaire. No missing cases occurred. Out of the 7263 students attending the first year of middle school in Torino, 3452 (47.5%) ate in the school cafeteria. Similarly, 184/400 (46.0%) of our random sample ate at the cafeteria. The parents were given the cafeteria menus, so they knew types and portions of the distributed food.

During the visit at the Sports Medicine Institute, a skilled dietician interviewed the participants in case of uncertain answers or discrepancy between data from the grid and the food-recall questionnaire, asking them to indicate how often per day and how many days a week they had usually consumed a specific food and its serving size with the aid of sample photos; 155/400 (38.8%) participants were interviewed. There was no significant difference in age, sex distribution, ethnicity, and BMI between the interviewed and non-interviewed participants. The food-recall questionnaire data were loaded on the software Win Food Pro 3 (Medimatica, Colonnella, Teramo, Italy). The estimated basal metabolic rate (BMR) for 10-18-year old individuals was calculated by the following formula in accordance with Schofield [28]: for males, BMR = 0.068 weight + 0.574 height + 2.157; for females, BMR = 0.035 weight + 1.948 height + 0.837. Then, the estimated energy requirement was calculated by multiplying the BMR by a coefficient corresponding to one of the three exercise levels (sedentary, moderately active, and active), in line with the Italian and European guidelines [29]. Finally, the reliability of the reported energy intake was assessed by calculating the ratio of estimated energy intake to predicted basal metabolic rate; subjects with a ratio <0.88 and >2.72 were classified as under-reporters or overreporters, respectively [30]: 12 under-reporters and 2 over-reporters were identified. Diet quality was evaluated with the simple dietary quality index (SDQI) score, first used in Denmark, comparing saturated fats (SFA) and fiber intake to the Danish dietetic recommendations [31]. An adjustment in the dietary fiber estimation was needed for our cohort because the Italian guidelines recommended 2g/MJ for children/adolescents. For each individual a score was calculated for SFA and dietary fiber, and the minimum and maximum scores for each nutrient were 0 and 100, respectively. The SDQI score was the mean of the scores for SFA and fiber; the highest was the score, the healthier was the diet [31].

Snacking episodes

Snacking episodes were defined as eating occasions not included in breakfast, lunch and dinner, in accord with the literature [8]. The snacking occasions were reported by the participants' parents,

along with a detailed description of the kind, amount, and time of the snacks. Morning snacking was considered as food consumption between breakfast and lunch, afternoon snacking between lunch and dinner, and evening snacking after dinner. Most participants consumed snacks in different occasions over the day. Individuals were categorized as predominantly morning, afternoon, and evening snackers if the highest caloric intake from snacks derived from the morning, afternoon, and evening snacks, respectively. Furthermore, analyses were repeated after considering snackers those whose daily caloric intake from snacks was ≥15% of the total daily caloric intake [13].

Statistical analysis

Based on available data [1,6], a sample size of 371 patients had a statistical power of 80% to detect a 2-fold higher prevalence of overweight/obesity in individuals consuming \geq 3 snacks/day, with a 2tailed α -value=0.05.

Normality distribution of data was checked by the Kolmogorov-Smirnov normally test. Exercise level, expressed as METs h/week, did not show a normality distribution, and differences among groups for this variable were analyzed by the Kruskal-Wallis test. Additionally, values were log-transformed, thus obtaining a normal distribution, and the log-transformed values were used in the regression model (see below).

The ANOVA and the χ^2 -test were performed to assess the differences in the continuous and categorical variables, respectively. A Poisson regression analysis with robust variance was used to estimate the relative risk of prevalent overweight/obesity by energy content, number, and timing of consuming the most energy-dense snack, after adjusting for age (continuous variable), sex (males=1), exercise levels (log-METs h/week), the SDQI score (continuous variable), and total caloric intake (continuous variable). The same analyses were replicated in the subgroup of participants who consumed $\geq 15\%$ of the total daily caloric intake as snacks (*n*=272).

Results

Table 1 summarizes the characteristics of the whole cohort of adolescents and selected sample undergoing the medical assessment. There was no difference between the adolescent cohort as a whole and the randomly selected group.

On average, participants consumed 1.9 ± 0.7 snacks/day (males: 1.9 ± 0.8 ; females: 2.0 ± 0.7), contributing to 18.1% of the daily energy intake. Most frequently consumed snacks were: sweets, savory snacks, milk/yogurt, fruit juice, and soft drinks; occasionally fruits, bread, cereals and grains; rarely fresh vegetables and cold cut.

The overall prevalence of overweight/obesity was 16.5% (66/400), and 29.5% of participants performed no exercise other than gymnastic class at school. The prevalence of overweight/obesity did not differ among individuals who ate at the cafeteria (16.4%) and those who did not (16.6%). Participants were divided according to the percentage of daily energy derived from snacks (Table 2). A low number of subjects (13/400; 3.3%) did not consume any snacks and showed lower total caloric intake, lower exercise levels, and a significantly higher prevalence of overweight/obesity. Notably, 6 of the 12 under-reporters were found in this category. To avoid the possibility that the association between no snacking and BMI may be due to reverse causality (i.e. subjects did not consume snacks because they were trying to lose weight), these individuals were excluded, and data from 387 participants were analyzed.

Increasing contribution of snacks to daily energy intake, from <15% to >20%, was associated with a significant increase in total caloric intake and a higher prevalence of overweight.

The number of snacks consumed during the day ranged form 1 to 5. The snack number (Table 3) was positively correlated with higher total caloric intake, reduced METs h/week, and increased overweight/obesity prevalence, varying from 9.6 to 22.6%. The increased caloric intake, however, was characterized by higher fiber and lower SFA intake.

Participants were also classified according to the timing of consuming the most caloric snack of the day (Table 4). In most cases, the morning (mean percentage of the total snack calories =63.9%) and the afternoon (64.4% of the snack calories) snacks showed the highest caloric content, while the

evening snack (69.3% of the snack calories) was the more calorie-dense in 28/387 cases only. The percentage of energy taken at breakfast, lunch, and dinner did not differ among morning, afternoon, and evening snackers (data not shown). The evening snackers showed increased fiber intake and a better SDQI score, but their overweight prevalence was high (28.6%), and in 39% of these individuals the evening snack was the only snack consumed during the day. Finally, the characteristics of the participants were evaluated by overweight/obesity status (Table 5): individuals with BMI $\ge 85^{\text{th}}$ percentile showed significantly higher SFA intake and lower fiber and SDQI score. In a Poisson regression model, the variables associated with BMI>85th percentile were fiber intake (RR=0.88; 95%CI 0.83-0.93; p < 0.001), SFA intake (RR=1.12; 95%CI 1.04-1.22; p=0.004), and the SDQI score, which is a combination of these two variables (RR=0.95; 95% CI 0.93-0.96; p < 0.001). In the same model, increased snacking calories and frequency and big evening snacks were significantly associated with BMI ≥85th percentile (Table 6). Analyses were repeated after considering only snackers who consumed $\geq 15\%$ total energy intake as snacks and after excluding the 6 under-reporters and the 2 over-reporters; the results did not change. Finally, frequency and timing of snacking were analyzed together in the same model, and both of them showed a positive association with overweight/obesity (RR=1.73; 95% CI 1.09-2.75; p=0.02 for consuming \geq 3 snacks/day; RR=1.98; 95%CI 1.18-3.33; p=0.01 for consuming the most caloric snack at evening).

Discussion

The calories, number and timing of snacks are associated with overweight/obesity, independent from diet quality and exercise frequency in a sample of 11-13-year old Italian adolescents.

Snacking is a common habit in adolescents: 97% of our subjects usually consumed at least one snack during the day, similarly to previous reports [9,14,32]. Snacks might represent a good source of fiber. If fruits and fresh vegetables were daily consumed as snacks, it would be easier to reach the recommended nutritional goals [8,32-33]. However, snacks generally fail to improve diet quality because they are usually energy-dense and high-carbohydrate foods, despite the fact they have a

lower fat content than main meals [8,11,32-33]. In our sample, adolescents with higher snacking frequency or quantity showed significantly higher fiber and total energy intake and lower SFA intake. Therefore, dietary quality, as evaluated by the SDQI score, was not lower in those who consumed the largest amount of calories as snacks (big snackers).

In our study, the prevalence of overweight/obesity was higher in participants consuming >20% calorie from snacks and \geq 3 snacks/day, as big snackers introduced more calories and were less active. It has been previously reported that usual snackers have higher total energy intake [4,6,8,34]. Prospective studies found a direct association between snacking and weight gain risk [3,6-7]. Snackers do not compensate for the energy intake excess by reducing caloric intake during the main meals [34]. Additionally, extra-eating occasions are thought to lead to excessive caloric intake because snacks might not generate extra-signals of satiety [35]. While a few studies found an increased vigorous activity in snackers [11,13], our results, consistent with most studies [3,5-6], show that those who consumed more or larger snacks were less physically active. Notably, the association between snacking and overweight/obesity remained significant after adjusting for exercise level and total energy intake.

Other studies found an inverse [11,14] or no association [10-13,36] between increased snacking and BMI or weight gain. To justify these findings, it has been hypothesized that splitting foods in multiple eating occasions resulted in reduced caloric intakes at each meal, and that increased eating frequency resulted in a reduction of insulin secretion [37]. However, the reference group for most studies was represented by those who did not consume any snacks [6,9,11] and had the higher BMI, similarly to our no-snacking-group. An explanation could be that overweight/obese individuals willing to lose weight avoided snacks or tended to under-report their food intakes, particularly snacks. The inverse relationship between BMI and meal frequency was no longer significant after excluding adolescents on calorie restriction diet [38]. To avoid the possibility of a systematic bias related to the reverse causality between high BMI and no snacking, we excluded from analyses individuals who had declared to skip snacks. Furthermore, a study evaluating energy expenditure by

whole-body calorimetry and doubly-labeled water tests did not demonstrate metabolic advantages of multiple eating occasions [38]. As the epidemiologic evidence of the inverse relationship between meal frequency and BMI is considered very weak, new controlled studies are needed [39]. Current conflicting findings may be also due to the lack of uniformity in the definition of snack [13].

Our most compelling result is the association between timing of the large snack and overweight. Only a few of our participants had the most caloric snack in the evening after dinner, and they showed the highest prevalence of overweight/obesity. It is interesting that a higher snacking frequency and the timing of consumption of the largest snacks were both independently associated with overweight/obesity in our participants. The actual trend for adolescents is to eat very little in the morning and to shift most daily caloric intake late [22]. When consumed in the afternoon or evening, food seems to induce a weaker sense of satiety, and larger servings tend to be consumed over the day [22]. As a consequence, late intakes are more likely to cause positive energy balance. A prospective study showed a significant positive effect of eating later in the day on BMI, suggesting a role of circadian variation in energy intake in the development of overweight [40]. These associations were confirmed in a large sample of US children [41]. Furthermore, diet-induced thermogenesis is reduced after consuming a snack in the evening compared to the morning and afternoon consumption; nocturnal insulin resistance or decreased whole body fat oxidation have been hypothesized to be responsible for the evening decline of the thermogenic response [20-21]. Insulin sensitivity decreases later in the day, and increased levels of free fatty acids and fluctuation in hormonal concentrations may be possible contributors to the circadian modulation of insulin secretion or action [42]. Additionally, morning gastric emptying may be more rapid than evening gastric emptying [43], and circadian variations in satiety hormones, energy expenditure, and genetic mutations of the circadian clock genes have been associated with weight gain and metabolic abnormalities in humans [44]. Finally, the evening snack could be consumed in front of the TV or

PC, a condition associated with a decrease in fruit and vegetables consumption and an increase in sweet beverage and energy-dense foods consumption [3,45].

Adolescence is a period of rapid hormonal variations and growth, potentially leading to profound nutritional changes; the dietary pattern established during this period generally doesn't change into adulthood, and overweight/obesity during adolescence might increase the risk of adult metabolic diseases. Therefore, it is important to define simple nutritional rules to help maintain healthy BMI. Defining the most convenient quantity, composition, and time of day of scheduled snacks in diets could be an adequate measure to improve adolescent lifestyle.

Limitations

There is no consensus on what constitutes a snack; our definition was based on a temporal criterion as most studies [8,11,32-33]. Indeed, adolescents in our age-range are yet conditioned by their parents in their time of eating. The food-based classification systems may be misleading, since many adolescents consumed sweets, fruit juice, and soft drinks as a part of their meals, and there are no foods/drinks consumed exclusively as snacks. Results did not change after considering as snackers subjects who consumed ≥15% of total energy intake as snacks. This is an observational study, and the possibility of residual confounding cannot be excluded. A 24h dietary recall may be inadequate to evaluate the individual usual eating pattern. However, it is usually employed for characterizing habits in groups of individuals [46], also in adolescents [4, 47-48]. Furthermore, we used the frequency grid as a help to check the plausibility of answers, and the participants themselves were interviewed by an experienced dietician in case of uncertain answers. In all of these cases, no considerable discrepancy was found between the measures reported by the parents and the interviewed participants.

We tried to check the accuracy of reporting and analyzed data after excluding under- and overreporters. Nevertheless, we could not exclude that these individuals were accurately reporting their caloric intake (e.g. they were overweight and may be trying to lose weight). The prevalence of overweight/obesity did not differ among individuals who ate at the cafeteria and those who did not; most of our participants were younger than 13 years (354/400), and the Centers for Disease Control and Prevention (CDC) has recently reported that purchasing sodas and snacks at school is much more frequent in individuals older than 12 years (3-fold higher in 13-15 years and 4-fold higher in 16-18 years) [49]. Finally, the comparability of the dietary intakes with the data from national surveys [50] and the strong inverse association between the SDQI score and overweight argued in favor of our data reliability. The study lacked statistical power to analyze the association between the kind of snacks consumed and overweight/obesity; furthermore, CI coefficients were quite large, indicating that there was variability in the association between snacking and weight status. Moreover, the majority of children who ate \geq 3 snacks or evening snacks were not overweight/obese thus suggesting that other protective factors (genetic, environmental, hormonal, etc.) need to be studied. Nevertheless, we think that our results on this controversial matter might be useful, and are worthy to be tested in larger studies.

The strengths of this study were the measurement of all the anthopometric variables by two trained researchers, as opposed to self-reported measures, and the finding that participants in the study were similar to all the adolescents living in Torino with the same age-range.

Conclusions

Snacking patterns are associated with overweight/obesity in adolescents. Circadian variation in energy intakes from snacks could be a modifiable factor worthy to be tested in obesity prevention or interventional trials.

Conflict of interest

The authors have no conflict of interest.

References

1) Larson N, Story M. A review of snacking patterns among children and adolescents: what are the implications of snacking for weight status? Child Obes 2013; 9: 104-115.

- 2) Binkin N, Fontana G, Lamberti A, et al. A national survey of the prevalence of childhood overweight and obesity in Italy. Obes Rev 2010; 11: 2-10.
- Francis LA, Lee Y, Birch LL. Parental weight status and girls' television viewing, snacking, and body mass indexes. Obes Res 2003; 11: 143-151.
- Nicklas TA, Yang SJ, Baranowski T, et al. Eating patterns and obesity in children. The Bogalusa Heart Study. Am J Prev Med 2003; 25: 9-16.
- 5) Lioret S, Touvier M, Lafay L, et al. Dietary and physical activity patterns in French children are related to overweight and socioeconomic status. J Nutr 2008; 138: 101-107.
- Bes-Rastrollo M, Sanchez-Villegas A, Basterra-Gortari FJ, et al. Prospective study of selfreported usual snacking and weight gain in a Mediterranean cohort: the SUN project. Clin Nutr 2010; 29: 323-330.
- Collison KS, Zaidi MZ, Subhani SN, et al. Sugar-sweetened carbonated beverage consumption correlates with BMI, waist circumference, and poor dietary choices in school children. BMC Publ Health 2010; 10: 234.
- Hampl JS, Heaton CLB, Taylor CA. Snacking patterns influence energy and nutrient intakes but not body mass index. J Hum Nutr Dietet 2003; 16: 3-11.
- Field AE, Austin SB, Gillman MW, et al. Snack food intake does not predict weight change among children and adolescents. Int J Obesity 2004; 28: 1210-1216.
- 10) Phillips SM, Bandini LG, Naumova EN, et al. Energy-dense snack food intake in adolescence: longitudinal relationship to weight and fatness. Obes Res 2004; 12: 461-472.
- 11) Keast DR, Nicklas TA, O'Neil CE. Snacking is associated with reduced risk of overweight and reduced abdominal obesity in adolescents: National Health and Nutrition Examination Survey (NHANES) 1999-2004. Am J Clin Nutr 2010; 92: 428-435.
- 12) Viskaal-van Dongen M, Kok FJ, de Graaf C. Effects of snack consumption for 8 weeks on energy intake and body weight. Int J Obes 2010; 34: 319-326.

- 13) Gregori D, Foltran F, Ghidina M, et al. The "snacking child" and its social network: some insights from an italian survey. Nutr J 2011; 10: 132.
- 14) Ritchie LD. Less frequent eating predicts greater BMI and waist circumference in female adolescents. Am J Clin Nutr 2012; 95: 290-296.
- 15) Wansink B, Shimizu M, Brumberg A. Association of nutrient-dense snack combinations with calories and vegetable intake. Pediatrics 2013; 131: 22-29.
- 16) Hastmann TJ, Bopp M, Fallon EA, et al. Factors influencing the implementation of organized physical activity and fruit and vegetable snacks in the HOP'N after-school obesity prevention program. J Nutr Educ Behav 2013; 45: 60-68.
- 17) Drummond S, Crombie N, Kirk T. A critique of the effects of snacking on body weight status. Eur J Clin Nutr 1996; 50: 779-783.
- 18) Ma Y, Bertone ER, Stanek III EJ, et al. Association between eating patterns and obesity in a free-living US adult population. Am J Epidemiol 2003; 158: 85-92.
- 19) Jennings A, Cassidy A, van Sluijs EM, et al. Associations between eating frequency, adiposity, diet, and activity in 9-10 year old healthy-weight and centrally obese children. Obesity 2012; 20: 1462-1468.
- 20) Romon M, Edme JL, Boulenguez C, et al. Circadian variation of diet-induced thermogenesis. Am J Clin Nutr 1993; 57: 476-480.
- 21) Hibi M, Masumoto A, Naito Y, et al. Nighttime snacking reduces whole body fat oxidation and increases LDL cholesterol in healthy young women. Am J Physiol Regul Integr Comp Physiol 2013; 304: R94-R101.
- 22) De Castro JM. When, how much and what foods are eaten are related to total daily food intake. Br J Nutr 2009; 102: 1228-1237.
- 23) Cacciari E, Milani S, Balsamo A, et al. Italian cross-sectional growth charts for height, weight and BMI (6-20 y). Eur J Clin Nutr 2002; 56: 171-180.

- 24) Ainsworth BE, Haskell WL, Herrrmann SD, et al. 2011 Compendium of physical activities: a second update of codes and MET values. Med Sci Sports Exerc 2011; 43: 1575-1581.
- 25) Pahkala K, Heinonen OJ, Lagström H, Hakala P, Sillanmäki L, Simell O. Leisure-time physical activity of 13-year-old adolescents. Scand J Med Sci Sports 2007; 17: 324-330.
- 26) Bo S, Ciccone G, Durazzo M, et al. Efficacy of antioxidant treatment in reducing resistin serum levels: a randomized study. PLoS Clin Trials 2007; e17.
- 27) Bo S, Bertino E, Trapani A, et al. Magnesium intake, glucose and insulin serum levels in pre-school very-low-birth weight pre-term children. Nutr Metab Cardiov Dis 2007; 17: 741-747.
- 28) Schofield WN. Predicting basal metabolic rate, new standards and review of previous work.Hum Nutr Clin Nutr 1985; 39 (Suppl 1): 5-41
- 29) http://www.sinu.it/documenti/20121016_LARN_bologna_sintesi_prefinale.pdf (in Italian)
- 30) Goldberg GR, Black AE, Jebb SA, et al. Critical evaluation of energy intake data using fundamental principles of energy physiology: 1. Derivation of cut-off limits to identify under-recording. Eur J Clin Nutr 1991; 45: 569-581.
- 31) Biltoft-Jensen A, Fagt S, Groth MV, et al. The intake of saturated fat and dietary fiber: a possible indicator of diet quality. Br J Nutr 2008; 100: 624-632.
- 32) Gatenby SJ. Eating frequency: methodological and dietary aspects. Br J Nutr 1997; 77: S7-S20.
- 33) Summerbell CD, Moody RC, Shanks J, et al. Sources of energy from meals versus snacks in220 people in four age groups. Eur J Clin Nutr 1995; 49: 33-41.
- 34) De Graaf C. Effects of snacks on energy intake: an evolutionary perspective. Appetite 2006;47: 18-23.
- 35) Marmonier C, Chapelot D, Fantino M, et al. Snacks consumed in a nonhungry state have poor satiating efficiency: influence of snack composition on substrate utilization and hunger. Am J Clin Nutr 2002; 76: 518-528.

- 36) Maffeis C, Grezzani A, Perrone L, et al. Could the savory taste of snacks be a further risk factor for overweight in children? J Pediat Gastr Nutr 2008
- 37) Jenkins DJ, Wolever TM, Vuksan V, et al. Nibbling versus gorging: metabolic advantages of increased meal frequency. N Engl J Med 1989; 321: 929-934.
- 38) Bellisle F, McDevitt R, Prentice AM. Meal frequency and energy balance. Br J Nutr 1997;77: S57-S70.
- 39) Mattson MP. The need for controlled studies of the effects of meal frequency on health.Lancet 2005; 365: 1978-1980.
- 40) Thompson OM, Ballew C, Resnicow K, et al. Dietary pattern as a predictor of change in BMI z-score among girls. Int J Obes 2006; 30: 176-182.
- 41) Eng S, Wagstaff DA, Kranz S. Eating late in the evening is associated with childhood obesity in some age groups but not in all children: the relationship between time of consumption and body weight status in U.S. Children. Int J Behav Nutr Phys Act 2009; 6: 27.
- 42) Van Cauter E, Desir D, Decoster C, et al. Nocturnal decrease of glucose tolerance during constant glucose infusion. J Clin Endocrinol Metab 1989; 69: 604-611.
- 43) Goo RH, Moore JG, Greenberg E, et al. Circadian variation in gastric emptying of meals in humans. Gastroenterology 1987; 93: 515-518.
- 44) Garaulet M, Gómez-Abellán P, Alburquerque-Bèjar JJ, et al. Timing of food intake predicts weight loss effectiveness. Int J Obes 2013; 37: 604-611.
- 45) De Craemer M, De Decker E, De Bourdeaudhuij I, et al. Correlates of energy balancerelated behaviours in preschool children: systematic review. Obes Rev 2012; 13: S13-S28.
- 46) Thompson FE, Byers T. Dietary assessment resource manual. J Nutr 1994; 124: 2245S-2317S.

- 47) Keast DR, Nicklas TA, O'Neil CE. Snacking is associated with reduced risk of overweight and reduced abdominal obesity in adolescents: National Health and Nutrition Examination Survey (NHANES) 1999-2004. Am J Clin Nutr 2010; 92: 428-435.
- 48) Slining MM, Mathias KC, Popkin BM. Trends in food and beverage source among US children and adolescents: 1989-2010. J Acad Nutr Diet 2013; 113: 1683-1694.
- 49) Mâsse LC, Blanck HM, Valente M, et al. Association between self-reported household practices and body mass index of US children and adolescents, 2005. Prev Chronic Dis 2012; 9: 110149.
- 50) Sebastian RS, Cleveland LE, Goldman JD. Effect of snacking frequency on adolescents' dietary intakes and meeting national recommendations. J Adolesc Health 2008; 42: 503-511.

	Sample studied	Cohort
Number	400	6876
Age (years)	12.1±0.5	11.7±0.7
Males (%)	52.0	52.5
Ethnicity (%)		
Caucasian	94.1	93.9
African	2.3	2.3
Asian	1.5	1.4
Hispanic	2.3	2.5
Metabolic equivalent of activity (METs h/week)	18.0 (31.5)	18.5 (32.0)
Weight (kg)	45.5±11.2	44.7±11.1
Height (m)	1.50±0.1	1.49±0.1
BMI (kg/m ²)	20.0±4.1	19.9 ±3.9

Table 1. Characteristics of the sample analyzed and of the whole cohort

Mean ±SD (all such values); median (inter-quartile range) (all such values)

BMI=body mass index

	0	<15%	15-20%	>20%
Number	13	115	111	161
Age (years)	12.0±0.4	12.2±0.5	12.1±0.5	12.0±0.6
Males (%)	61.5	49.6	54.1	51.6
Metabolic equivalent of activity* (METs h/week)	17.0 (38.0)	18.0 (29.0)	22.0 (42.0)	16.0 (27.0)
Weight (kg)	47.8±11.3	45.6±11.4	45.1±10.0	45.6±12.1
Height (m)	1.50±0.1	1.51±0.1	1.50±0.1	1.50±0.1
BMI (kg/m ²)	21.1±4.0	19.8±3.9	19.9±3.4	19.9±4.3
BMI percentile*	53.0 (56.0)	40.0 (44.0)	40.0 (62.0)	47.0 (62.0)
BMI≥85 th percentile(%)	38.5	10.4	14.4	20.5
Total Kcal	1866.6±424.9	1943.3±347.9	1961.8±481.7	2104.6±461.7#
HC(% cal)	51.1±16.2	51.3±15.8	51.0±13.8	50.7±13.4
Fat (% cal)	35.1±10.1	35.4±10.7	36.0±10.1	36.0±9.6
SFA (% cal)	10.9±3.0	11.0±3.0	10.9±3.7	10.5±3.0
Protein(% cal)	13.8±3.7	13.3±3.8	13.0±4.0	13.4±3.9
Fiber(g/day)	13.3±4.0	16.2±6.6	15.9±6.0	15.8±5.6
SDQI score	71.4±14.7	74.2±13.7	73.0±15.2	73.8±14.2

Table 2. Characteristics of the participants by percentage of daily energy intake from snacks

Mean ±SD (all such values); median (inter-quartile range) (all such values); BMI=body mass index; BP=blood pressure;

HC=carbohydrates; SFA= saturated fatty acids; SDQI= simple dietary quality index; *Kruskal-Wallis analysis; #p<0.01

	1	2	>2
	1	L	≥3
Number	83	220	84
Age (years)	12.1±0.4	12.1±0.5	12.1±0.7
Males (%)	54.2	50.5	52.4
Metabolic equivalent of activity* (METs h/week)	21.0 (33.0)	20.0 (34.0)	15.0 (23.0)§
Weight (kg)	43.4±10.7	45.9±10.9	46.3±12.7
Height (m)	1.50±0.1	1.51±0.1	1.50±0.1
BMI (kg/m ²)	19.1±3.7	20.1±3.8	20.4±5.0
BMI percentile*	26.0 (39.0)	49.5 (60.0)	54.5 (64.5)#
BMI≥85 th percentile(%)	9.6	15.5	22.6
Total kcal	1851.7±397.8	1978.6±391.1	2274.9±502.7§
HC (% cal)	51.0±13.8	51.1±14.8	51.3±15.2
Fat (% cal)	36.0±10.4	35.8±10.0	35.5±10.2
SFA (%/cal)	11.3±3.5	10.8±3.3	10.1±2.2#
Protein (% cal)	13.0±3.8	13.1±4.0	13.2±3.9
Fibre (g/day)	14.3±5.2	16.3±6.3	16.6±5.6#
SDQI score	70.9±13.9	74.3±15.5	74.9±11.1

Table 3. Characteristics of the participants by number of snacks consumed per day

Mean ±SD (all such values); median (inter-quartile range) (all such values); BMI=body mass index; BP=blood pressure;

HC=carbohydrates; SFA= saturated fatty acids; SDQI= simple dietary quality index; *Kruskal-Wallis analysis; \$p<0.05; #p<0.01

		Morning snack	Afternoon snack	Evening snack
Number		180	179	28
Age (years)		12.1±0.4	12.1±0.6	12.2±0.7
Males (%)		55.0	48.6	50.0
Metabolic equivalent of activity* (METs h/week)	16.0 (35.5)	18.0 (31.0)	19.0 (26.0)
Number snacks/day				
	1	15.6	24.6	39.3
	≥2	84.4	75.4	60.7#
Weight (kg)		45.7±11.0	44.9±11.2	47.2±13.1
Height (m)		1.51±0.1	1.50±0.1	1.49±0.1
BMI (kg/m ²)		19.9±3.9	19.8±4.0	21.2±5.6
BMI percentile*		32.5 (59.0)	45.0 (59.0)	50.0 (57.5)§
BMI≥85 th percentile (%)		13.3	16.2	28.6
Total kcal		1990.8±443.5	2028.7±446.9	2092.5±411.0
HC (% cal)		51.4±15.7	51.0±13.4	51.6±15.1
Fat (% cal)		35.3±10.8	35.9±9.8	35.2±10.3
SFA (% cal)		10.9±3.4	10.6±3.0	10.4±2.6
Protein (% cal)		13.3±3.8	13.1±3.6	13.2±3.8
Fiber (g/day)		15.1±5.9	16.9±6.3	16.0±3.8#
SDQI score		71.6±15.2	75.5±13.7	75.3±10.8#

 Table 4. Characteristics of the participants by the timing of consuming the most energy-dense snack

Mean ±SD (all such values); median (inter-quartile range) (all such values); BMI=body mass index; BP=blood pressure;

HC=carbohydrates; SFA= saturated fatty acids; SDQI= simple dietary quality index; *Kruskal-Wallis analysis; \$p<0.05; #p<0.01

	BMI≥85 th percentile	BMI<85 th percent	
Number	61	326	
Age (years)	12.1±0.6	12.1±0.5	
Males (%)	57.4	50.6	
Metabolic equivalent of activity* METs h/week)	15.0 (34.0)	18.0 (31.0)	
Weight (kg)	62.4±9.1	42.3±8.4#	
Height (m)	1.52±0.1	1.50±0.1	
BMI (kg/m^2)	27.1±2.9	18.6±2.6#	
BMI percentile*	95.0 (10.0)	28.0 (47.0)#	
Predicted Kcal	2036.2±387.4	1971.5±304.0	
Fotal Kcal	2015.7±532.5	2015.3±424.7	
IC(% cal)	50.9±14.2	51.3±14.5	
Fat (% cal)	36.0±10.1	35.5±10.2	
SFA (% cal)	11.8±3.9	10.5±2.9#	
Protein(% cal)	13.4±3.7	13.3±3.8	
Fiber (g/day)	13.0±4.1	16.5±6.1#	
SDQI score	63.8±15.4	75.5±13.4#	

Table 5. Characteristics of the participants by BMI percentiles

Mean ±SD (all such values); median (inter-quartile range) (all such values); BMI=body mass index; BP=blood pressure;

HC=carbohydrates; SFA= saturated fatty acids; SDQI= simple dietary quality index; *Kruskal-Wallis analysis; \$p<0.05; #p<0.01

Table 6. Association between BMI≥85th percentile and the energy content, number, and

		RR	95% CI		Р
% energy intake from snacks*					
	15-20%	1.35	0.58	3.15	0.49
	>20%	2.32	1.10	4.89	0.03
Males		1.26	0.70	2.29	0.44
SDQI score		0.95	0.93	0.96	< 0.001
Metabolic equivalent of activity**		0.88	0.66	1.17	0.36
Number of snacks***					
	2	2.20	0.92	5.27	0.08
	≥3	4.17	1.60	10.9	0.004
Males		1.28	0.70	2.32	0.42
SDQI score		0.94	0.92	0.96	< 0.001
Metabolic equivalent of activity**		0.89	0.67	1.19	0.44
Timing of the most energy-dense snack****					
	Afternoon	1.08	0.57	2.03	0.82
	Evening	3.12	1.17	8.34	0.02
Males		1.30	0.72	2.35	0.39
SDQI score		0.94	0.92	0.96	< 0.001
Metabolic equivalent of activity**		0.89	0.67	1.18	0.42

timing of consuming the most energy-dense snack in a Poisson regression analysis

SDQI=simple dietary quality index

*the controls were individuals consuming <15% daily energy from snacks

** Metabolic equivalent of activity (METs h/week) were log-transformed to obtain a normal distribution

***the controls were individuals consuming 1 snack

****the controls were individuals consuming the most energy-dense snack in the morning All analyses were adjusted for age and total caloric intake.