European Journal of Pediatrics
https://doi.org/10.1007/s00431-021-04064-5

ORIGINAL ARTICLE

Reduction of small dense LDL and Il-6 after intervention with Plantago psyllium in adolescents with obesity: a parallel, double blind, randomized clinical trial

Alma Patricia González[1,2] · Anaisa Flores-Ramírez[1] · Karla Paola Gutiérrez-Castro[1] · Claudia Luévano-Contreras[1] · Armando Gómez-Ojeda[1] · Gloria Patricia Sosa-Bustamante[2] · Russell Caccavello[3] · Juan Carlos Barrera-de León[4,5] · Ma Eugenia Garay-Sevilla[1] · Alejandro Gugliucci[3]

Received: 20 October 2020 / Revised: 26 March 2021 / Accepted: 4 April 2021

Abstract

Obesity can lead children and adolescents to an increased cardiovascular disease (CVD) risk. A diet supplemented with Plantago psyllium has been shown to be effective in reducing LDL-C and IL-6 in adolescents. However, there are no studies that have explored small-dense LDL (sdLDL) or HDL subclasses. The aim of this study was to evaluate the impact of a fiber dietary intervention on LDL and HDL subclasses in adolescents with obesity. In this parallel, double blind, randomized clinical trial, the participants were assigned to Plantago psyllium or placebo (10g/day for 7 weeks). We randomized 113 participants, and evaluated and analyzed 100 adolescents (50 in each group), 15 to 19 years with a body mass index of 29–34. We measured biochemical markers LDL and HDL subclasses using the Lipoprint system (Quantimetrix) and IL-6 by ELISA. Post-treatment there was a decrease in sdLDL between the groups 2.0 (0–5.0) vs 1 (0–3.0) mg/dl ($p = 0.004$), IL-6 median 3.32 (1.24–5.96) vs 1.76 (0.54–3.28) pg/ml, $p < 0.0001$. There were no differences in HDL subclasses and no adverse effects were reported in either group.

Conclusions: Small dense LDL and IL-6 reduced in adolescents with obesity when consuming Plantago psyllium. This may be an early good strategy for the reduction of cardiovascular disease risk in this vulnerable population.

Trial registration: ISRCTN # 14180431. Date assigned 24/08/2020

Communicated by Peter de Winter

✉ Ma Eugenia Garay-Sevilla
marugaray_2000@yahoo.com

✉ Alejandro Gugliucci
alejandro.gugliucci@tu.edu

Alma Patricia González
patipili999@hotmail.com

Anaisa Flores-Ramírez
anaisa_390@hotmail.com

Karla Paola Gutiérrez-Castro
paola1_castro@hotmail.com

Claudia Luévano-Contreras
c.luevanocontreras@ugto.mx

Armando Gómez-Ojeda
gomez.ojeda.armando@gmail.com

Gloria Patricia Sosa-Bustamante
gloria.sosa@imss.gob.mx

Russell Caccavello
russell.caccavello@tu.edu

Juan Carlos Barrera-de León
juan-barrerale@imss.gob.mx

[1] Department of Medical Science, Division of Health Science, University of Guanajuato, Campus León, Guanajuato, Mexico

[2] High Specialty Medical Unit, Hospital of Gynecology and Pediatrics # 48, Mexican Institute of Social Security, León, Mexico

[3] Glycation, Oxidation and Disease Laboratory, Dept. of Research, College of Osteopathic Medicine, Touro University California, Vallejo, CA, USA

[4] Western National Medical Center, Mexican Institute of Social Security, Guadalajara, Mexico

[5] University of Guadalajara, Guadalajara, Mexico

Published online: 16 April 2021

What is Known:
• *Supplementing the diet with Plantago psyllium lowers LDL-C levels.*
What is New:
• *First evidence that soluble fiber supplementation like Plantago psyllium decreases small dense LDL particles in association with lowered IL-6, reducing the risk of cardiovascular disease in obese adolescents.*

Keywords Obesity · Adolescents · sdLDL · IL-6 · *psyllium* · Cardiovascular risk · MetS

Introduction

The prevalence of obesity in infants, children, and adolescents is rising around the world [1]. Mexico has the highest prevalence of obesity documented globally [2]. Excess body fat in children and adolescents can lead to a variety of clinical and psychosocial disorders such as nonalcoholic fatty liver disease, type 2 diabetes, cardiovascular disease, hypercholesterolemia, glucose intolerance, and insulin resistance among other diseases [3].

Low-density lipoprotein cholesterol (LDL-C) is considered one of the most important risk factors for cardiovascular disease (CVD) and remains the primary target for current cardiovascular risk reduction strategies [4]. However, many individuals with LDL-C within the normal range still develop cardiovascular disease and this is known as the residual risk, which may be as high as 50% [5]. A systematic review and meta-analysis in adults of general vs. high-risk populations suggests that the presence of sdLDL is associated with increased risk of developing coronary heart disease [6]. Mechanistically, sdLDL increase the atherogenic risk due to their greater susceptibility to oxidation and glycation, their permeability through the endothelium of arterial walls, and the fact that they derive from large VLDL1 which are associated with high carbohydrate diets [4, 7]. There is very limited information on sdLDL in adolescents. Miyashita M et al. and ourselves have showed that the sdLDL was present in 40% and 63%, respectively, of the adolescents with obesity [8, 9]. Intervention studies in adults using omega-3 polyunsaturated fatty acids, or with nutraceuticals agents, have shown a decrease in sdLDL particles [10].

HDL is highly heterogeneous in size, charge, and composition which are modified during HDL maturation from small to large HDL. The HDL particles are usually divided into three subclasses (using Lipoprint) based on their size: small (S-HDL), medium (M-HDL), and large (L-HDL). Antiatherogenic large HDL subfractions and intermediate HDL subfractions are at present considered a protective part of HDL and small HDL subfractions are deemed atherogenic [11]. Mietus-Snyder ML et al. showed that consumption of high-fiber, nutrient-dense supplement bar, increased large HDL subfraction in healthy adults [12]. However, there have been a limited number of studies on HDL subfractions in adolescents [11, 13].

Epidemiologically, insufficient consumption of dietary fiber has been associated with the frequency of chronic diseases [14]. Adolescents consume less than one half of the recommended adequate intake of dietary fiber which is around 8 g/day [15]. A meta-analysis of randomized controlled trials showed that the consumption of dietary fiber reduce the risk of cardiovascular disease [16]. On the other hand, reports in adolescents have shown that supplementing the diet with *Plantago psyllium* lowers LDL-C levels [17, 18]. Studies indicate an inverse association between dietary fiber intake and levels of inflammatory markers such as C-reactive protein, tumor necrosis factor-alpha, and interleukin 6 (IL-6) [14, 19, 20]. To our knowledge, there are no reports of the effect of the Plantago psyllium on sdLDL or HDL subfractions in adolescents, and it is known that an accurate assessment of cardiovascular risk factors and an intervention from an early age play a decisive role in CVD prevention. Therefore, we hypothesized that supplementing the diet with *Plantago psyllium* exerts beneficial effects on sdLDL, HDL subclasses, and IL-6 in adolescents with obesity.

Materials and methods

Ethics approval

Ethics approval for this study was provided by the Locals Ethics Committees [University of Guanajuato (CIBIUG-P40-2017) and the Mexican Institute of Social Security, (R-2018-1002-052)] and written informed consent was obtained from participants and caregivers in compliance with ethical standards. ISRCTN # 14180431.

Subjects

Adolescents with obesity between 15 and 19 years old were recruited from high schools in León Guanajuato (México) to participate in the study (Fig. 1). Inclusion criteria were at least one cardiovascular risk factor (altered lipid profile, either high total cholesterol, high triglycerides, or low HDL-C, and insulin resistance) and non-smokers. Exclusion criteria were

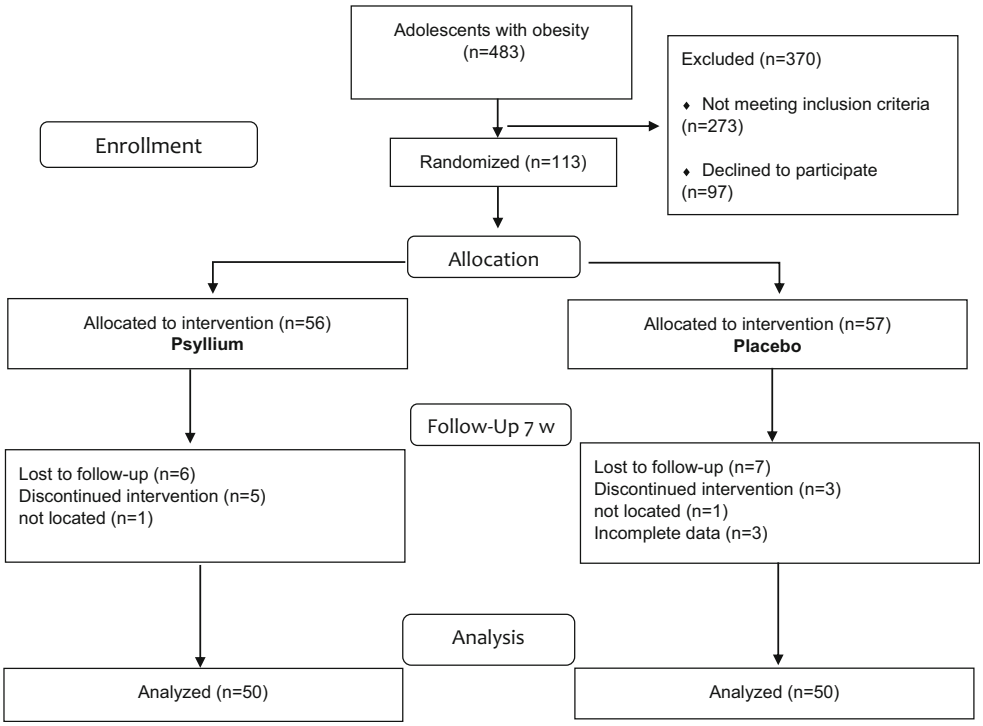

Fig. 1 CONSORT 2010 flow diagram

adolescents who did not adhere to at least 80% of diet and fiber, in whom a metabolic and/or infectious disease was diagnosed during the study and adolescents with familial hypercholesterolemia based on LDL-C ≥160 mg/dl and a first-degree relative with LDL-C or with premature CVD or with positive genetic testing [21]. We eliminated those who did not want to continue in the study or were not located.

Sample size

Randomization order was established before the recruitment of participants, a minimum sample size of one hundred subjects (i.e., fifty in each group) as required by the 80% power calculation was aimed for to detect a change of above 5% in sdLDL concentrations [22] in the treatment groups with a significance level of 0.05.

Study design

Recruitment began on November 2018 and the study ended on December 2019. The study was a parallel, double blind, randomized clinical trial, (participant and investigator who gave the treatment) placebo-controlled trial. A research randomization and allocation to trial group were done using computer random number generation. All participants were randomly assigned to one of two groups for the principal research: the psyllium group with 7-week intervention with 10 g/day of psyllium (equating to 10 g of dietary fiber), *Plantago psyllium* powder (Kirkland signature ®, Lot 0294B12) and the placebo group with 7-week intervention with 10 g/day of rice flour (Healthy Flours ® batch AB140119) both were diluted in 250 ml of water, ingested immediately after dilution with intake of 250 ml of additional water, in the morning and before ingesting food (Fig. 1). The dose of 10 g/day was adopted based on review of the existing literature[23–25], as well as on the volume of fiber and placebo each dose would equate to, so as not to affect compliance with study protocol. Both the *psyllium* and rice flour were packed in opaque bags labelled A or B.

Each dose was given daily by the blinded investigator who visited the adolescents in the morning from Monday through Friday, and during the weekend, the consumption was verified through a photograph sent by the participant. Adherence to dosing was monitored directly through a check list and by WhatsApp. Participants were advised to continue their normal eating and exercise patterns during the study period. At each visit during the dietary treatment phase, the participants were asked about possible adverse effects or intolerance to *psyllium* or placebo using an open-ended questionnaire referring to any unusual symptoms or discomfort or side effects such as increased defecation, bloating, flatulence, or fullness during the treatment period.

Dietary assessment

Three 24-h food reminders were carried out at the beginning (2 on school days and one on a weekend day) and three reminders at the end of the intervention. Both recalls (baseline and post-treatment) were analyzed with ESHA's Food Processor® Nutrition Analysis software. The software

determined the average initial and final energy intake and we analyzed the two averages. Nutritional intake was recorded using standard household measures, as well as the information from food labels where appropriate.

Anthropometric measures

The height and weight of the child were measured using a SECA stadiometer and a SECA scales, respectively, and body mass index (BMI) was calculated as kilogram per square meter (kg/m^2), waist circumference (WC) was measured using Lufkin ® metallic tape, blood pressure was measured with an Omron HEM-7320-LA electronic monitor (Omron Healthcare Co. Ltd, Kyoto, Japan) after 10 minutes of the non-dominant hand while the subject was sitting, and the average of 3 measurements was recorded. Along with scores and centiles, which was scored using the definitions set by the Centre for Disease Control and Prevention were taken according to standardized techniques [26] and adolescents with obesity were considered as a BMI for age more than 2 standard deviations above the median established in the World Health Organization (WHO) Child Growth Standards[1]. The atherogenic index (AI) (total cholesterol/HDL-C) was calculated [27]. The evaluation of anthropometric measures and biochemical parameters in the participants was carried out twice at baseline and at the end of the study.

Biochemical analyses

A venous blood sample was obtained after 12 h of fasting. Serum was processed the same day and used for the measurement of glucose and lipids using enzymatic methods in an autoanalyzer (Spinreact-Spinlab, Model 6002390-412-02). Serum aliquots were stored at −80 °C until further determination of insulin, by ELISA kit 06026 from ALPCO (Salem, NH, USA) and Interleukin 6 by Human IL-6 Quantikine ELISA Kit, Catalogue number HS600B (USA and Canada) [cut-off point to IL-6, 0.98±0.33] [28]. LDL and HDL subclasses were analyzed in serum by gradient electrophoresis in polyacrylamide gel tubes by using the Lipoprint LDL and HDL subfraction analyses systems from Quantimetrix (Redondo Beach, CA, USA), which measures lipid concentration in each fraction and defines seven subclasses of LDL (LDL 1–7) and ten subclasses of HDL (HDL 1–10) [29] and due to the relatively large volume of serum needed to measure HDL subclasses (50 ul), we randomly run out of serum in some of the participants and only 34 were measured in the Plantago psyllium group and 30 in the placebo group.

Statistical analysis

The distribution and normality of each variable was determined by the Kolmogorov Smirnov test. Data are shown as mean ± SD for variables with normal distribution and the difference between groups was calculated using t tests for independent sample or as median and interquartile range (IQR) for non-Gaussian distribution and the difference was calculated using Mann-Whitney *U* test. To assess the effects of BMI, waist circumference, and LDL-C, in outcome variables in the placebo and *Plantago psyllium* group, we used a mixed linear model. A significance level of <0.05 was considered. The statistical package IBM SPSS (SPSS Inc., Chicago, IL, USA) statistics version 25 was used.

Results

A flow diagram depicting the progress of participants in the trial is shown in Fig. 1. The randomization of the study participants was carried out regardless of their metabolic status. However, at the beginning of the study, the groups were similar, adolescents with insulin resistance (IR) were 45 (90%) on the psyllium group and 48 (96%) on the placebo group, as well as 26 participants (48%) had elevated LDL-C in both groups. The baseline analysis revealed that both the control and *Plantago psyllium* groups had similar anthropometric indices, biochemical variables. No significant differences were observed between the control and *Plantago psyllium* groups (p>0.05) (Table 1).

The changes observed in the general characteristics at baseline and post-treatment groups are shown in Table 2. The analysis shows that after 7 weeks of treatment, both groups showed increased in height (as expected) as well as a decrease in total cholesterol and LDL-C. Besides, we detected a significant decrease in body mass index, waist circumference in the *Plantago psyllium* group as well as total cholesterol, non-HDL-C, and LDL-C. There was a marginal decrease in the atherogenic index. The placebo group showed lower levels of LDL-C, non-HDL-C, and total cholesterol at the end of treatment as shown in Table 2.

The results of the mixed linear model to observe the influence of the variables BMI, waist circumference, LDL-C, HDL-C, total cholesterol, and triglycerides on the IL-6, sdLDL and HDL lipoprotein subfractions shown as estimate ± SE and p value are as follows: significant influence was observed in LDL-1 of LDL-C (−0.06±0.05, p=0.02) and HDL-C (0.91±0.42, p=0.03); LDL-2 of LDL-C (0.10±0.04, p=0.008); large-HDL of BMI (1.09±0.40, p=0.009) and waist circumference (−0.40±0.14, p=0.006); HDL-Intermediate with BMI (−0.63±0.029, p=0.03); and waist circumference (0.25±0.10, p=0.02); no significant influence is shown on other dependent variables such as IL-6 or sdLDL 3-7 and small HDL (Supplementary Table)

The analysis of LDL subfractions is detailed in Table 3 where it is shown that, in the comparison between the baseline vs the post-treatment values, a decrease in LDL-2 (p=0.04) and sdLDL

Table 1 Baseline characteristics of the participants

Variables	*Plantago psyllium* $n = 50$	Placebo $n = 50$	p
Female (%)**	35 (64.8)	31 (60.7)	0.76
Age (years)*	16 (15.0–17.2)	16 (15.0–17.0)	0.57
Weight (kg)*	87.8 (80.6–97.50)	84.0 (76.7–93.3)	0.13
Height (m)	1.66 (1.59–1.72)	1.62 (1.58–1.67)	0.23
Body mass index (kg/m^2)*	31.8(29.8–34.7)	31.0 (29.5–33.3)	0.38
Waist circumference (cm)	100.4 ± 12.5	98.2 ± 9.0	0.31
Systolic blood pressure (mmHg)*	112.0 (105.0–120.0)	110..0(100.0–120.0)	0.37
Diastolic blood pressure(mmHg)*	77.0 (70.0–80.0)	74.5 (70.0–80.0)	0.21
Glucose (mg/dl)*	94.5 (89.0–100.2)	95.0 (88.0–101.0)	0.96
Insulin (μUI/ml) *	28. 0(20.7–42.1)	26.1(20.1–44.0)	0.91
HOMA IR *	7.4(4.6–11.0)	6.6 (5.1–10.4)	0.98
Cholesterol (mg/dl)	175.9 ± 32.8	171.8 ± 34.5	0.54
No HDL-C (mg/dl)	133.0 ± 30.5	132.7 ± 36.2	0.73
HDL-C (mg/dl)*	39.5 (35.0–45.0)	38.0 (34.0–41.0)	0.23
LDL-C (mg/dl)	112.1 ± 26.7	107.6 ± 29.6	0.42
VLDL-C (mg/dl)*	23.5(17.0–29.0)	24.5 (17.7–32.2)	0.39
Triglycerides (mg/dl)*	120.5 (84.5–154.0)	120.5 (90.0–167.5)	0.57
Atherogenic index (Total Cl/HDL-C)*	4.3 (3.9–4.9)	4.1 (3.8–5.5)	0.85

Data are shown as mean ± SD for variables with normal distribution and the difference between groups was calculated using t tests for independent or as median and interquartile range for non-Gaussian distribution and the difference was calculated using Mann-Whitney U test.*, and to compare percentages, we use x^{2} ** No HDL-C (No HDL cholesterol), HDL-C (high-density lipoproteins), LDL-C (low-density lipoproteins), VLDL-C (very low-density lipoproteins), HOMA (homeostatic model to assess insulin resistance)

(p=0.004) was found in the group that received *Plantago psyllium*; and when comparing the sdLDL particles concentration between the groups after the 7-week treatment, a greater decrease was observed in the adolescents who consumed *Plantago psyllium* (p=0.01) and the percentage of change was −40.38%.

Table 4 shows the distribution of the 10 HDL subfractions in 34 and 30 adolescents who received *Plantago psyllium* or placebo, respectively, and we did not observe significant difference in any of the subfractions in either group.

Figure 2 shows that the *Plantago psyllium* group had a significant decrease of IL-6 (in picograms per milliliter) post-treatment with a median of 3.32 (1.24–5.96) vs 1.76 (0.54–3.28), p <0.00001; no difference in the placebo group median 2.10 (1.07–4.10) vs 2.28 (1.22–4.36), p=0.58, nor in the comparison between the groups baseline *Plantago psyllium* vs placebo group median 3.32(1.24–5.96) vs 2.10 (1.07–4.10) respectively, p=0.05, and post-treatment *Plantago psyllium* group median 1.76 (0.54–3.28) vs placebo group median 2.28 (1.22–4.36), p=0.09.

Discussion

This is the first clinical trial conducted in adolescents with obesity that shows the effect of *Plantago psyllium* on emerging cardiovascular risk markers and shows that it significantly decreases the levels of sdLDL and IL-6. Several studies have shown that soluble dietary fiber reduces LDL cholesterol [17, 18, 24, 25, 30], but there were no reports to our knowledge on the effect of *Plantago psyllium* on sdLDL in adolescents with obesity.

Interestingly, we show significant decrease in LDL-C in both groups even when the participants were following their usual diet. Participants were only given dietary recommendations, without modifying the amounts or restricting calories. In agreement with this, we found no difference in energy intake, or in total fiber or soluble fiber intake, before or after the intervention. An explanation for the decrease in LDL-C in both groups could be due to observation bias: participants slightly altered their diet. Small-dense LDL is highly atherogenic subfraction of LDL (LDL-3 to LDL-7 in Lipoprint) due to their long plasma half-life, lower binding affinity for LDL receptors, greater degree of penetration into the arterial wall, and sensitivity to oxidative stress and glycation [4]. Studies have shown that sdLDL particles are found 3 times more often in healthy adults than in children, and people with this phenotype have a three times greater risk of myocardial infarction [31]. SdLDL is a better predictor of CVD than traditional lipid profiles [4, 32]. Indeed higher larger fractions of LDL (LDL-1 and LDL-2 in Lipoprint) are not atherogenic. Moreover,

Table 2 Comparison of the groups at baseline and post-treatment

Variables	Plantago psyllium *n*=50			Placebo *n*=50		
	Baseline	Post-treatment	p	Baseline	Post-treatment	*p*
Anthropometric variables						
Body mass index (kg/m^2)	31.8(29.8–34.7)	31.3 (29.3–34.1)	0.002	31.0 (29.5–33.5)	31.0 (29.7–33.1)	0.17
Waist circumference (cm)	98.5 (95.0–107.2)	98.0 (91.0–102.0)	0.003	97.5 (92.0–104.0)	97.5 (92.0–103.0)	0.94
Systolic blood pressure (mmHg)	112.0 (105.0–120.0)	110.0 (103.0–116.0)	0.06	110..0(100.0–120.0)	109 (99.0–118.0)	0.33
Diastolic blood pressure (mmHg)	77.0 (70.0–80.0)	75.0 (70.0–80.0)	0.11	74.5 (70–80.0)	74.0(69.0–81.0)	0.28
Dietary variables						
Energy (Kcal/day)	2465.7 ± 477.6	2385.5 ± 417.5	0.41	2403.6 ± 321.5	2453.0 ± 390.4	0.06
Proteins (g/day)	93.9 ± 28.3	93.9 ± 28.3	0.58	91.6 ± 14.4	93.7 ± 22.8	0.50
Carbohydrates (g/day)*	295.3 (257.4–350.1)	322.9 (253.3–367.0)	0.70	303.62 (261.0–367.0)	290.42 (248,3–328.14)	0.35
Total fat (%)	32.36 ± 7.37	33.45 ± 6.65	0.93	33.14 ± 6.43	33.94 ± 6.48	0.41
Sat fat (%)	9.88 ± 3.60	10.25 ± 3. 43	0.31	10.39 ± 3.43	10.82 ± 3.65	0.71
Total fiber (g/day)*	15.3 (9.9–21.3)	15.5 (11.3–21.6)	0.92	14.8 (10.6–18.8)	13.4 (10.3–17.0)	0.21
Soluble fiber (g/day)*	2.8 (1.5–4.2)	2.7 (1.6–4.7)	0.85	2.5 (1.6–3.7)	2.5 (1.2–3.7)	0.36
Metabolic variables						
Glucose (mg/dl)*	93.5 (89.0–100.0)	92.0 (87–100.0)	0.37	95.0 (89.0–101.0)	95.0 (84–102.0)	0.99
Insulin (μ IU/ml)*	28.0(21.0–42.1)	26.6(18.6–39.6)	0.12	26.1(20.1–44.0)	25.3 (19.9–36.10)	0.07
HOMA IR*	7.4(4.6–11.0)	6.4(4.3–9.1)	0.06	6.6 (5.1–10.4)	6.3 (5.0–8.5)	0.12
Total cholesterol (mg/dl)	175.9 ± 32.8	160.5 ± 31.4	0.001	171.8 ± 34.5	162.9 ± 45.8	0.01
No HDL-C (mg/dl)	135.0 ± 30.6	135.1 ±30.6	0.001	132.7 ± 36.2	125.5 ± 46.6	0.01
HDL-C (mg/dl)*	39.5 (35.0–45.0)	39.0(33.0–44.0)	0.08	38.0 (34.0–41.2)	36.5 (31.0–41.0)	0.09
LDL-C (mg/dl)	112.1 ± 26.7	97.8 ± 25.3	0.001	107.6 ± 29.6	97.1 ± 26.4	0.001
VLDL-C (mg/dl)*	23.5(17.0–29.0)	24.5 (18.0–31.0)	0.25	4.5 (18–35)	26.5 (19–36.0)	0.14
Triglycerides (mg/dl)*	120.5 (85–0–154.0)	122.5 (88.0–153.2)	0.22	120.5 (91.0–165.0)	132.0(93–179.0)	0.23
Atherogenic index (total C/HDL-C)*	4.3 (3.9–4.9)	4.2 (3.4–4.8)	0.05	4.1 (3.8–5.4)	4.35 (3.5–5.5)	0.26

Data are shown as mean ± SD for variables with normal distribution and the difference between groups was calculated using paired t tests for variables with normal distribution or data expressed as median and interquartile range for non-Gaussian distribution and was used Wilcoxon test.*No HDL-C (no HDL cholesterol), HDL-C (high-density lipoproteins), LDL-C (low-density lipoproteins), VLDL-C (very low-density lipoproteins)

sdLDL stems from large VLDL1 which are highly sensitive to carbohydrates (CHO) in the diet, especially sugars [33]. Our participants are consumers of these diets high in processed foods. Of note, we observed a high prevalence of sdLDL (64%), consistent with the previous study of our research group [9]. We show a higher proportion than Miyashita

Table 3 LDL subclasses expressed as mg/dl of cholesterol at baseline and post-treatment (after 7 weeks) in the participants

Plantago psyllium *n*=50				Placebo *n*=50			
	Baseline	Post-treatment	*p**	Baseline	Post-treatment	*p**	*p***
LDL 1	34.0 (28–42.0)	33.0 (2.0–40.0)	0.08	31.0 (23–38.0)	28.0(21–0–36.06)	0.70	0.21
LDL 2	22.38 ± 9.94	19.84 ± 8.95	0.04	19.52 ± 8.37	18.52 ± 9.33	0.96	0.47
LDL 3–7 (sdLDL)	2.0 (0–5.0)	1 (0–3.0)	0.004	2 (0–6.0)	2 (1.0–5.0)	0.64	0.01

Data are shown as mean ± SD for variables with normal distribution and the difference between groups was calculated using *t*-tests or as median and interquartile range for non-Gaussian distribution and was used Mann-Whitney *U* test/Wilcoxon test

*Baseline vs. post-treatment *p* value of the group, **post-treatment *p* value between the groups. *LDL* low-density lipoproteins; *sdLDL* small dense low-density lipoproteins

Table 4 Comparison of the 10 high-density lipoprotein subfractions expressed as mg/dl of cholesterol at the baseline and post-treatment of the participants

		Plantago psyllium *n*=34			Placebo *n*=30			
		Baseline	Post-treatment	*p*°	Baseline	Post-treatment	*p*°	*p*°°
Large	L-HDL 1	6.0 (4.0–9.0)	6.0 (4.0–8.00)	0.42	5.0 (4.0–8.0)	5.0 (4–7)	0.81	0.25
	L-HDL 2	8.0(5.0–11)	8.9 (5.0–11.0)	0.21	7.0(6.0–12.0)	8.0(5.0–11.0)	0.94	0.90
	L-HDL 3	5.0(3.0–6.0)	5.0(3.0–5.0)	0.45	5.0 (4.0–6.0)	4.0(3.0–6.0)	0.83	0.93
Intermediate	I-HDL 4 *	6.21 ± 1.26	6.18 ± 1.33	0.88	6.07 ± 1.99	5.64 ± 1.83)	0.20	0.23
	I-HDL 5 *	6.45 ± 1.41	6.63 ± 1.38	0.50	6.29 ± 2.13	5.93 ± 2.11	0.13	0.11
	I-HDL 6 *	5.78 ± 2.47	6.18 ± 2.06	0.39	5.54 ± 2.26	5.16 ± 2.39	0.20	0.09
	I-HDL 7 *	1.24 ± 1.09)	1.24 ± 0.93	1.0	1.06± 0.92)	0.93 ± 0.9	0.29	0.15
Small	S-HDL 8	1.0 (0–1)	1.0 (0–1)	1.0	0 (0–1)	0 (0–1)	0.61	0.48
	S-HDL 9	0 (0–1)	0 (0–1)	0.80	0 (0–1)	0 (0–1)	0.52	0.41
	S-HDL 10	0 (0–0)	0 (0–0)	0.31	0 (0–0)	0 (0–0)	0.89	0.93

The difference between groups was calculated using *t* tests* for normally distributed variables or the Mann-Whitney U/Wilcoxon test for non-Gaussian distribution variables

HDL high density lipoproteins; large HDL (L-HDL 1 to L-HDL 3), intermediate HDL (I-HDL 4 to I-HDL 7), and small HDL (S-HDL 8 to S-HDL 10)

p° Compare the baseline vs post-treatment of each group

p°° Compare the difference post-treatment between the groups

Unfortunately, due to the relatively large volume of serum needed to measure subclasses (50 ul), we run out of serum in some of the participants\

et al. [8] who found that 40% of obese children with lipid abnormalities had sdLDL particles, most likely due to much lower sugar consumption in Japan, which also underscores the magnitude of the issue in Mexico.

It has been documented that the ingestion of *Plantago psyllium* significantly reduces LDL cholesterol in adults (10.2g/d/≥3weks) [23] and adolescents (6–7g/d/7weeks) [17, 18, 24]. Along the same lines, we observed that using 10 g per day of *Plantago psyllium* for 7 weeks, sdLDL reduced by 40.38%. The exact mechanism by which *Plantago psyllium* lowers cholesterol is not yet fully understood. So far, evidence suggests that some soluble fibers bind to bile acids or cholesterol during intraluminal micelle formation [23, 34, 35]. The resulting reduction in the cholesterol content of the liver cells leads to upregulation of the hepatic LDL receptors and thus increases the elimination of LDL-C. Moreover, (1) soluble fiber changes microbiota and increases short-chain fatty acids (SCFAs) that increase liver insulin sensitivity; (2) it binds CHO producing slower release to portal circulation. Both effects reduce lipogenesis [new fatty acid (FA) from CHO] and

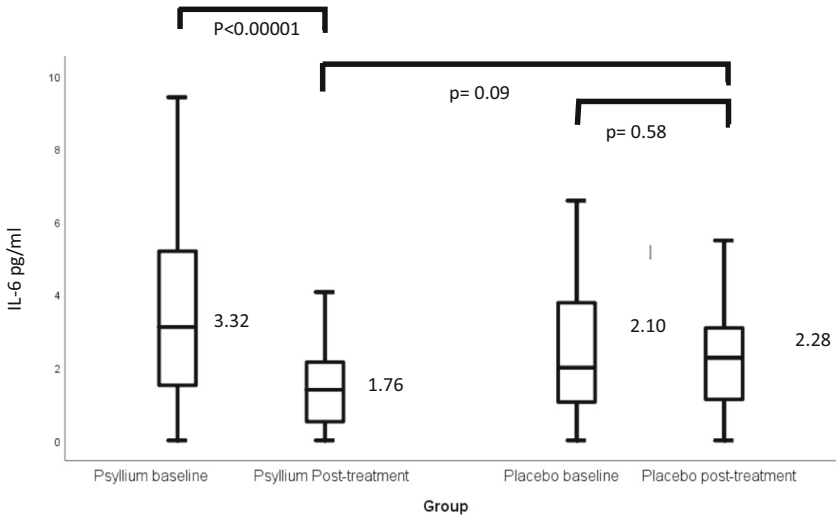

Fig. 2 Serum levels of interleukin 6 at baseline and after treatment in adolescents with obesity

re-esterification of FA into triglycerides [36, 37]. The result is less VLDL1 secretion, which is the one that carries more apoCIII and is less metabolized by lipoprotein lipase (LPL). Consequently, its lipolysis by hepatic and endothelial lipases is enhanced, leading to higher sdLDL levels. Since sdLDL is not the predominant fraction (but it is the highly atherogenic one), small changes in total LDL-C are indeed hiding large fractional changes in sdLDL, in addition to increased satiety, leading to a lower total energy intake [38].

Like LDL, HDL particles are heterogeneous, and HDL subclasses play interrelated metabolic functions. Changes in HDL subclasses distribution might be linked with atherosclerosis. Small HDL (HDL-8 to HDL-10 in Lipoprint) particles are considered atherogenic [11]. The small subfractions of HDL could have a diminished ability to protect LDL from oxidation, attenuated RCT, as well as remnant reverse cholesterol transport (RRCT) and anti-inflammatory properties, i.e., impaired functionality [39, 40]. Therefore, we had reasoned that reducing these particles with *Plantago psyllium* may improve HDL functionality. We found that 53% of adolescents had S-HDL, but we did not find a significant difference between the groups. There is limited information on these particles as markers of cardiovascular risk in adults [40, 41], and few reports that show that children and adolescents with obesity have high levels of small HDL [42, 43].

In adults, controversial data are reported; on the one hand, there is no difference after administering soluble fiber as oats 14 g/days/for 7 weeks in large HDL [44], and on the other hand, an increase is reported in large HDL particles after administering bar soluble and insoluble fiber 14g/days/for 2 weeks [12]. It has been suggested that an increased cholesteryl ester transfer protein (CETP) activity and a diminished lecithin cholesterol acyltransferase (LCAT) activity are associated with variation of HDL subclasses distribution. A study reported that *Plantago psyllium* supplementation significantly decreased CETP activity [45], suggesting that psyllium indirectly affects the intravascular processing of lipoproteins by reducing the transfer of cholesteryl ester from HDL to VLDL. Nevertheless, in our cohort, we did not observe a statistically significant difference in HDL subclasses after treatment.

Obesity can be considered a low-grade inflammatory disease, characterized by high plasma levels of pro-inflammatory cytokines such as IL-6, among others. Studies show the ability of IL-6 to induce insulin resistance (IR); in fact, elevated levels of IL-6 are considered risk indicators for metabolic syndrome; since as an inducer of inflammation, it seems to be related both to lipid and vascular disturbances found in IR [46]. Our study showed that adolescents had elevated IL-6 levels as well as insulin resistance based on HOMA-IR results. In agreement with our study, another group of researchers showed that adolescents with obesity and IR presented subclinical systemic inflammation by presenting levels of IL-6 almost twice that of adolescents without IR, and IL-6 was positively associated with all indicators of adiposity [42, 43]. We observed a decrease in IL-6 at the end of treatment in the group that received psyllium; therefore, our findings confirm previous reports and suggest that a higher intake of soluble fiber may contribute to reducing IL-6 and simultaneously decrease insulin resistance, in addition to reducing IL-6, which can lead to the reduction of chronic low-grade inflammation and improve health outcomes in adolescents.

Early insulin resistance is a prominent feature of obesity that promotes chronic systemic inflammation and increased oxidative stress [42] increasing NF kappa B signaling pathway and subsequent inflammatory events [47].

After 7 weeks of treatment, we observed a significant decrease in adiposity indices such as waist circumference and BMI, and there was a trend to a decrease in HOMA-IR in the *Plantago psyllium* group. The mechanisms underlying the effects of soluble fiber consumption on insulin resistance can be attributed to the fermentation process with the consequent production of SCFA [48], to the prebiotic effect that influences the composition of the microbiota favoring bacterial species that do not trigger endotoxemia (i.e., lipopolysaccharides and peptidoglycans) [49] and a modulation of the expression of glucose transporter type 4 (GLUT-4) in skeletal muscle, thus improving insulin sensitivity [50, 51].

This study shows several strengths; (i) to our knowledge, this is the first study to show the distribution of HDL in adolescents with obesity and the effect of *Plantago psyllium* on emerging risk markers such as sdLDL-C and (ii) the study participants received equal dietary recommendations in the intervention groups towards a healthy diet and there was no modification in caloric intake. A limitation of these kinds of studies is that full control of the diet cannot be achieved as the participants continue with their usual diet.

Conclusions

In conclusion, our study showed that in adolescents with obesity and cardiovascular risk, *Plantago psyllium* supplementation decreased cardiovascular risk markers sdLDL and IL-6; therefore, it can be a good strategy for the reduction of this risk in adolescents, a vulnerable and critical period of development where dietary intervention may result in timely and long term beneficial effects.

List of Abbreviations *AI,* Atherogenic index; *BMI,* Body mass index; *CETP,* Cholesteryl ester transfer protein; *CHO,* Carbohydrates; *CVD,* Cardiovascular disease; *FA,* Fatty acid; *GLUT-4,* Glucose transporter type 4; *HDL,* High-density lipoproteins; *IL-6,* Interleukin-6; *IQR,* Interquartile range; *IR,* Insulin resistance; *LCAT,* Lecithin cholesterol acyltransferase; *LDL,* Low-density lipoprotein; *RRCT,* Remnant reverse cholesterol transport; *SCFA,* Short chain fatty acids; *SdLDL,* Small dense low density lipoprotein; *VLDL,* Very low-density lipoproteins

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00431-021-04064-5.

Author's contributions All authors contributed to the study conception and design; material preparation, data collection, and analysis were performed by Alma Patricia González, Anaisa Flores Ramírez, Karla Paola Gutiérrez Castro, Claudia Luévano Contreras, Russell Caccavello, Ma Eugenia Garay-Sevilla, and Alejandro Gugliucci]; the first draft of the manuscript was written by Alma Patricia González mentored by Ma Eugenia Garay-Sevilla and Alejandro Gugliucci, and all authors gave their input. All authors read and approved the final manuscript.

Funding University of Guanajuato (DAIP 211/2019 and 195/2020), Mining Company Santa María de la Paz, SLP, México, Touro-University California, Vallejo Ca. USA.

Availability of data and material Data and material available by request to Ma. Eugenia Garay-Sevilla (megaray@ugto.mx).

Code availability Not applicable.

Declarations

Ethics approval Ethics approval for this study was provided by the Locals Ethics Committees [University of Guanajuato (CIBIUG-P40-2017) and the Mexican Institute of Social Security, (R-2018-1002-052)] ISRCTN # 14180431.

Consent to participate Written informed consent was obtained from the parents, and from all individual participants included in the study.

Consent for publication All adolescents and their parents gave consent for the publication of their data anonymously if is necessary.

Conflicts of interest The authors declare no competing interests.

References

1. World Health O (2017) Report of the Commission on Ending Childhood Obesity: implementation plan: executive summary World Health. World Health Organization. License: CC BY-NC-SA 3.0 IGO. https://apps.who.int/iris/handle/10665/259349. Accessed 31 May 2017
2. Duffey KJ, Rivera JA, Popkin BM (2014) Snacking is prevalent in Mexico. J Nutr 144:1843–1849. https://doi.org/10.3945/jn.114.198192
3. Aceves-Martins M, Llauradó E, Tarro L, Solà R, Giralt M (2016) Obesity-promoting factors in Mexican children and adolescents: challenges and opportunities. Glob Health Action 9:1–13. https://doi.org/10.3402/gha.v9.29625
4. Hoogeveen RC, Gaubatz JW, Sun W, Dodge RC, Crosby JR, Jiang J, Couper D, Virani SS, Kathiresan S, Boerwinkle E, Ballantyne CM (2014) Small dense LDL cholesterol concentrations predict risk for coronary heart disease: the Atherosclerosis Risk in Communities (ARIC) Study. Arterioscler Thromb Vasc Biol 34:1069–1077. https://doi.org/10.1161/ATVBAHA.114.303284
5. Collins R, Armitage J, Parish S, Sleight P, Peto R (2002) MRC/BHF Heart Protection Study of cholesterol lowering with simvastatin in 20 536 high-risk individuals: a randomised placebo-controlled trial. Lancet 360:7–22. https://doi.org/10.1016/S0140-6736(02)09327-3
6. Liou L, Kaptoge S (2020) Association of small, dense LDL-cholesterol concentration and lipoprotein particle characteristics with coronary heart disease: a systematic review and meta-analysis. PLoS One 15:1–16. https://doi.org/10.1371/journal.pone.0241993
7. Ivanova EA, Myasoedova VA, Melnichenko AA, Grechko AV (2017) Orekhov AN (2017) Small dense low-density lipoprotein as biomarker for atherosclerotic diseases. Oxidative Med Cell Longev 2017:1–10. https://doi.org/10.1155/2017/1273042
8. Miyashita M, Okada T, Kuromori Y, Harada K (2006) LDL particle size, fat distribution and insulin resistance in obese children. Eur J Clin Nutr 60:416–420. https://doi.org/10.1038/sj.ejcn.1602333
9. Rodríguez-Mortera R, Luevano-Contreras C, Solorio-Meza S, Caccavello R, Bains Y, Garay-Sevilla ME, Gugliucci A (2018) Higher D-lactate levels are associated with higher prevalence of small dense low-density lipoprotein in obese adolescents. Clin Chem Lab Med 56:1100–1108. https://doi.org/10.1515/cclm-2017-0733
10. Talebi S, Bagherniya M, Atkin SL, Askari G, Orafai HM, Sahebkar A (2020) The beneficial effects of nutraceuticals and natural products on small dense LDL levels, LDL particle number and LDL particle size: A clinical review. Lipids Health Dis 19:1–21. https://doi.org/10.1186/s12944-020-01250-6
11. Muchová J, Andrezálová L, Oravec S, Nagyová Z, Garaiova I, Duracková Z (2016) High density lipoprotein subfractions and paraoxonase 1 in children. Acta Biochim Pol 63:555–563. https://doi.org/10.18388/abp.2015_1230
12. Mietus-Snyder ML, Shigenaga MK, Suh JH, Shenvi SV, Lal A, McHugh T, Olson D, Lilienstein J, Krauss RM, Gildengoren G, McCann JC, Ames BN (2012) A nutrient-dense, high-fiber, fruit-based supplement bar increases HDL cholesterol, particularly large HDL, lowers homocysteine, and raises glutathione in a 2-wk trial. FASEB J 26:3515–3527. https://doi.org/10.1096/fj.11-201558
13. Barbora K, Magdaléna V, Iveta W, Stanislav O, Iveta G, Zuzana N, Nataša H, Zdenka Ď, Jana T (2020) Lipid profile, lipoprotein subfractions, and fluidity of membranes in children and adolescents with depressive disorder: effect of omega-3 fatty acids in a double-blind randomized controlled study. Biomolecules 10:1–19. https://doi.org/10.3390/biom10101427
14. Parikh S, Pollock NK, Bhagatwala J, Guo DH, Gutin B, Zhu H, Dong Y (2012) Adolescent fiber consumption is associated with visceral fat and inflammatory markers. J Clin Endocrinol Metab 97:1451–1457. https://doi.org/10.1210/jc.2012-1784
15. Barquera S, Rivera JA, Safdie M, Flores M, Campos-Nonato I, Campirano F (2003) Energy and nutrient intake in preschool and school age Mexican children: National Nutrition Survey 1999. Salud Publica Mex 45:540–550. https://doi.org/10.1590/s0036-36342003001000010
16. McRae MP (2017) Dietary fiber is beneficial for the prevention of cardiovascular disease: an umbrella review of meta-analyses. J Chiropr Med 16:289–299. https://doi.org/10.1016/j.jcm.2017.05.005
17. Ribas SA, Cunha DB, Sichieri R, Santana Da Silva LC (2015) Effects of psyllium on LDL-cholesterol concentrations in Brazilian children and adolescents: a randomised, placebo-controlled, parallel clinical trial. Br J Nutr 113:134–141. https://doi.org/10.1017/S0007114514003419
18. de Bock M, Derraik JGB, Brennan CM, Biggs JB, Smith GC, Cameron-Smith D, Wall CR, Cutfield WS (2012) Psyllium supplementation in adolescents improves fat distribution & lipid profile: a randomized, participant-blinded, placebo-controlled, crossover trial. PLoS One 7:e41735. https://doi.org/10.1371/journal.pone.0041735

19. Swann OG, Breslin M, Kilpatrick M, O'Sullivan TA, Mori TA, Beilin LJ, Oddy WH (2020) Dietary fibre intake and its association with inflammatory markers in adolescents. Br J Nutr 125:329–336. https://doi.org/10.1017/S0007114520001609
20. Lottenberg AMP, Fan PLT, Buonacorso V (2010) Effects of dietary fiber intake on inflammation in chronic diseases. Einstein (São Paulo) 8:254–258. https://doi.org/10.1590/s1679-45082010md1310
21. Maliachova O, Stabouli S (2018) Familial hypercholesterolemia in children and adolescents: diagnosis and treatment. Curr Pharm Des 24:3672–3677. https://doi.org/10.2174/1381612824666181010145807
22. Garoufi A, Vorre S, Soldatou A, Tsentidis C, Kossiva L, Drakatos A, Marmarinos A, Gourgiotis D (2014) Plant sterols-enriched diet decreases small, dense LDL-cholesterol levels in children with hypercholesterolemia: A prospective study. Ital J Pediatr 40:1–6. https://doi.org/10.1186/1824-7288-40-42
23. Jovanovski E, Yashpal S, Komishon A, Zurbau A, Mejia SB, Ho HVT, Li D, Sievenpiper J, Duvnjak L, Vuksan V (2018) Effect of psyllium (Plantago ovata) fiber on LDL cholesterol and alternative lipid targets, non-HDL cholesterol and apolipoprotein B: a systematic review and meta-analysis of randomized controlled trials. Am J Clin Nutr 108:922–932. https://doi.org/10.1093/ajcn/nqy115
24. Moreno LA, Tresaco B, Bueno G, Fleta J, Rodríguez G, Garagorri JM, Bueno M (2003) Psyllium fibre and the metabolic control of obese children and adolescents. J Physiol Biochem 59:235–242. https://doi.org/10.1007/BF03179920
25. Cicero AFG, Colletti A, Bajraktari G, Descamps O, Djuric DM, Ezhov M, Fras Z, Katsiki N, Langlois M, Latkovskis G, Panagiotakos DB, Paragh G, Mikhailidis DP, Mitchenko O, Paulweber B, Pella D, Pitsavos C, Reiner Ž, Ray KK, Rizzo M, Sahebkar A, Serban MC, Sperling LS, Toth PP, Vinereanu D, Vrablík M, Wong ND, Banach M (2017) Lipid lowering nutraceuticals in clinical practice: Position paper from an International Lipid Expert Panel. Arch Med Sci 13:965–1005. https://doi.org/10.5114/aoms.2017.69326
26. Riley M, Hernandez AK, Kuznia AL (2018) High blood pressure in children and adolescents. Am Fam Physician 98:486–494
27. Rodríguez-Morán M, Guerrero-Romero F, Aradillas-García C, Bermudez-Peña C, Simental-Mendia LE, Vargas Morales JM, Torres Rodríguez ML, De La Cruz Mendoza E (2013) Atherogenic indices and prehypertension in obese and non-obese children. Diab Vasc Dis Res 10:17–24. https://doi.org/10.1177/1479164112440713
28. Takumansang R, Warouw SM, Lestari H (2013) Interleukin-6 and insulin resistance in obese adolescents. Paediatr Indones 53:268. https://doi.org/10.14238/pi53.5.2013.268-72
29. Hoefner DM, Hodel SD, O'Brien JF, Branum EL, Sun D, Meissner I, McConnell JP (2001) Development of a rapid, quantitative method for LDL subfractionation with use of the quantimetrix lipoprint LDL system. Clin Chem 47:266–274. https://doi.org/10.1093/clinchem/47.2.266
30. Xiao Z, Chen H, Zhang Y, Deng H, Wang KW, Bhagavathula AS, Almuhairi SJ, Ryan PM, Rahmani J, Dang M, Kontogiannis V, Vick A, Wei Y (2020) The effect of psyllium consumption on weight, body mass index, lipid profile, and glucose metabolism in diabetic patients: a systematic review and dose-response meta-analysis of randomized controlled trials. Phytother Res 34:1237–1247. https://doi.org/10.1002/ptr.6609
31. Austin MA, Breslow JL, Hennekens CH, Buring JE, Willett WC, Krauss RM (1988) Low-density lipoprotein subclass patterns and risk of myocardial infarction. JAMA 260:1917–1921. https://doi.org/10.1001/jama.1988.03410130125037
32. Zaki Khalil RMA, Al-Azab DAM, Akl OA (2017) Is sdLDL a valuable screening tool for cardiovascular disease in patients with metabolic syndrome? Alexandria J Med 53:299–305. https://doi.org/10.1016/j.ajme.2017.01.002
33. Packard CJ, Boren J, Taskinen MR (2020) Causes and consequences of hypertriglyceridemia. Front Endocrinol (Lausanne) 11:1–15. https://doi.org/10.3389/fendo.2020.00252
34. Anderson JW (1987) Dietary fiber, lipids and atherosclerosis. Am J Cardiol 60:G17–G22. https://doi.org/10.1016/0002-9149(87)90587-X
35. National Research Council (US) Committee on Diet and Health (1989) Diet and health: implications for reducing chronic disease risk. National Academies Press (US), Washington (DC)
36. Nishina PM, Freedland RA (1990) The effects of dietary fiber feeding on cholesterol metabolism in rats. J Nutr 120:800–805. https://doi.org/10.1093/jn/120.7.800
37. Schneeman BO, Gallaher D (1985) Effects of dietary fiber on digestive enzyme activity and bile acids in the small intestine. Proc Soc Exp Biol Med 180:409–414. https://doi.org/10.3181/00379727-180-42197
38. Slavin J, Green H (2007) Dietary fibre and satiety. Nutr Bull 32:32–42. https://doi.org/10.1111/j.1467-3010.2007.00603.x
39. Kontush A, Chapman MJ (2006) Functionally defective high-density lipoprotein: a new therapeutic target at the crossroads of dyslipidemia, inflammation, and atherosclerosis. Pharmacol Rev 58:342–374. https://doi.org/10.1124/pr.58.3.1
40. Oravec S, Dukat A, Gavornik P, Kucera M, Gruber K, Gaspar L, Rizzo M, Toth PP, Mikhailidis DP, Banach M (2014) Atherogenic versus non-atherogenic lipoprotein profiles in healthy individuals. Is There a need to change our approach to diagnosing dyslipidemia? Curr Med Chem 21:2892–2901. https://doi.org/10.2174/09298673216614030315304 8
41. Zeljkovic A, Vekic J, Spasojevic-Kalimanovska V, Jelic-Ivanovic Z, Bogavac-Stanojevic N, Gulan B, Spasic S (2010) LDL and HDL subclasses in acute ischemic stroke: prediction of risk and short-term mortality. Atherosclerosis 210:548–554. https://doi.org/10.1016/j.atherosclerosis.2009.11.040
42. Lopez-Sandoval J, Sanchez-Enriquez S, Rivera-Leon EA, Bastidas-Ramirez BE, Garcia-Garcia MR, Gonzalez-Hita ME (2018) Cardiovascular risk factors in adolescents: Role of insulin resistance and obesity. Acta Endocrinol 14:330–337. https://doi.org/10.4183/aeb.2018.330
43. Pires A, Martins P, Pereira AM, Silva PV, Marinho J, Marques M, Castela E, Sena C, Seiça R (2015) Insulin resistance, dyslipidemia and cardiovascular changes in a group of obese children. Arq Bras Cardiol 104:266–273. https://doi.org/10.5935/abc.20140206
44. Davy BM, Davy KP, Ho RC, Beske SD, Davrath LR, Melby CL (2002) High-fiber oat cereal compared with wheat cereal consumption favorably alters LDL-cholesterol subclass and particle numbers in middle-aged and older men. Am J Clin Nutr 76:351–358. https://doi.org/10.1093/ajcn/76.2.351
45. Vega-López S, Vidal-Quintanar RL, Femandez ML (2001) Sex and hormonal status influence plasma lipid responses to psyllium. Am J Clin Nutr 74:435–441. https://doi.org/10.1093/ajcn/74.4.435
46. Marcos-Gómez B, Bustos M, Prieto J, Martínez JA, Moreno-Aliaga MJ (2008) Obesity, inflammation and insulin resistance: Role of gp 130 receptor ligands. An Sist Sanit Navar 31:113–123
47. Negre-Salvayre A, Dousset N, Ferretti G, Bacchetti T, Curatola G, Salvayre R (2006) Antioxidant and cytoprotective properties of high-density lipoproteins in vascular cells. Free Radic Biol Med 41:1031–1040. https://doi.org/10.1016/j.freeradbiomed.2006.07.006
48. González Hernández MA, Canfora EE, Jocken JWE, Blaak EE (2019) The short-chain fatty acid acetate in body weight control and insulin sensitivity. Nutrients 11. https://doi.org/10.3390/nu11081943
49. da Silva ST, dos Santos CA, Bressan J (2013) Intestinal microbiota; relevance to obesity and modulation by prebiotics and probiotics.

Nutr Hosp 28:1039–1048. https://doi.org/10.3305/nh.2013.28.4.6525

50. Song YJ, Sawamura M, Ikeda K, Igawa S, Yamori Y (2000) Soluble dietary fibre improves insulin sensitivity by increasing muscle GLUT-4 content in stroke-prone spontaneously hypertensive rats. Clin Exp Pharmacol Physiol 27:41–45. https://doi.org/10.1046/j.1440-1681.2000.03198.x

51. Bozzetto L, Costabile G, Della Pepa G, Ciciola P, Vetrani C, Vitale M, Rivellese AA, Annuzzi G (2018) Dietary fibre as a unifying remedy for the whole spectrum of obesity-associated cardiovascular risk. Nutrients 10. https://doi.org/10.3390/nu10070943