JONNPR. 2016;1(7):244-253
DOI: 10.19230/jonnpr.2016.1.7.1087

Journal
of Negative & No Positive Results

Original
Artículo inglés

A study of anthropometric, biochemical and nutritional parameters in adolescents in the area of Toledo, Spain.

Características antropométricas, bioquímicas y nutricionales en una muestra de adolescentes del Estudio Área de Toledo.

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Abstract
Background. An increase of cardiovascular disease (CVD) risk in the younger population has been reported in Spain. Adolescents have changed their dietary habits and increased the risk of metabolic syndrome (MS). The Longitudinal study in the area of Toledo aims to evaluate food intake and nutritional habits and their relationship with early development of CVD and insulin resistance/sensitivity biomarkers.
Methods. A cross-sectional study was performed on 53 adolescents aged 16-17 years belonging to the Area of Toledo. Energy, macronutrient and micronutrient intakes and diet quality were assessed, as well as the prevalence to being overweight, the presence of CVD risk factors and parameters related to glucose homeostasis to identify candidates for MS.
Results. Adolescents consumed monotonous diets, with low Mediterranean diet Adherence (MDA) and Health Eating Index (HEI) scores and with elevated energy contribution of saturated fatty acids and low carbohydrates. Being overweight but not obese was moderately prevalent in volunteers. However, a low percentage of them showed dyslipemia or insulin resistance. No significant differences between male and female adolescents were found for any dietary parameter tested. HEI and MDA scores appear inversely related to insulin resistance markers in boys and to fat mass in girls, respectively.
Conclusions. The high similitude of diet in both male and female subjects suggests a general adherence to potentially negative dietary habits in this population. Paradoxically, dietary components and altered lipoprotein factors were not related. The overweight prevalence and, the association between diet quality and MS-markers found demands a follow-up study to ascertain the importance of present results later in life.

KEYWORDS
Adolescents; diet; insulin resistance; cardiovascular disease markers; metabolic syndrome.

Resumen
Antecedentes: En España se han reportado incrementos en algunos factores de riesgo cardiovascular. Los adolescentes, entre otros, han cambiado sus hábitos alimentarios y se ha constatado un aumento del riesgo de padecer síndrome metabólico (SM). El estudio longitudinal Área de Toledo tiene como objetivo evaluar la ingesta de alimentos y los hábitos nutricionales y su relación con el desarrollo temprano de la enfermedad cardiovascular (ECV) y la resistencia a la insulina / biomarcadores de sensibilidad.
Métodos: Estudio transversal que se realizó en un 53 adolescentes de 16-17 años pertenecientes al estudio Área de Toledo. Se evaluó la ingesta energética, de macro y micronutrientes así como de calidad de la dieta, la prevalencia de sobrepeso y la presencia de factores y parámetros relacionados con la homeostasis de la glucosa para identificar candidatos para MS y riesgo de ECV.
Resultados: Los adolescentes consumen dietas monótonas, con baja adherencia a la puntuación del Índice de Alimentación Saludable (IES) y a la dieta mediterránea (MDA), y con el aporte de energía elevada de ácidos grasos saturados y baja de hidratos de carbono. E 20% de los adolescentes tenían sobrepeso, pero se mostró un bajo riesgo de ECV y se objetivó una sensibilidad a la insulina aceptable. No se encontraron diferencias significativas entre hombres y mujeres adolescentes para cualquier parámetro de la

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Recibido el 10 de septiembre de 2016; aceptado el 18 de septiembre de 2016.

DOI: 10.19230/jonnpr.2016.1.7.1087

dieta evaluados. Las puntuaciones del IES tuvieron relación inversa con marcadores de resistencia a la insulina en los varones, mientras que la puntuación de la MDA con la masa grasa en las chicas.
Conclusiones: La alta similitud de las niñas y los niños respecto al tipo de dietas sugiere una adhesión general a los posibles hábitos alimentarios negativos en esta población. Paradójicamente, componentes de la dieta y los factores alterados de lipoproteínas no estaban relacionados. La alta prevalencia de sobrepeso relativa y las asociaciones entre la calidad de la dieta y marcadores de MS encontrados exigen un estudio de seguimiento para determinar la importancia de presentar los resultados más adelante en la vida.

PALABRAS CLAVE
Adolescentes; dieta; síndrome metabólico; marcadores de riesgo cardiovascular.

Introduction:

Atherosclerosis remains the main cause of morbidity and mobility all over the World with Spain scoring among countries with the lowest global and cardiovascular disease (CVD) mortality rates[1]. Improvement in primary prevention seems related[2]. However, during the last few decades, CVD risk incrementation has occurred in Spanish children and adolescents. The situation has become even worse on coastal Mediterranean provinces, traditionally showing low CVD rates and high Mediterranean diet adherence (MDA)[3-6]. Thus, the incidence and prevalence of child obesity and dyslipidemia, among other factors, has increased in Andalusia and the Canary Islands on several ocasions, while Madrid and the central provinces display the lowest rates[4,6]. Changes in dietary pattern (e.g. decrease in complex carbohydrates and increase in saturated fats) and physical activity have been reported[5,7]. Moreover, the present Spanish diet[7-9] is frequently lacking in mineral and vitamin Recommended Dietary Intakes[10] or present Dietary Reference Intakes[11].

The Mediterranean diet has been considered one of the healthiest diets as it is associated with a low prevalence of major degenerative diseases[12-14]. It is rich in vegetable foods and moderately rich in fish, poor in red meat. Olive oil is almost unique as a culinary oil[14,15]. This food combination assures a proper fatty acid profile and a large content of vitamins, bioactive and antioxidant compounds. However, reduction in the MDA in Spain and other Mediterranean countries[16] has been reported as well as potential health benefits[13,14,17,18].

Based on the previous premise, the present paper aims to study a sample of adolescents in the area of Toledo, Spain, in order to ascertain: a) their dietary characteristics, b) their anthropometrical values, lipoproteins and CVD ratios, insulin ensitivity/resistance markers and c) to ascertain possible relationships between those parameters in order to design, if needed, primary intervention to reduce future CVD and Metabolic Syndrome risks.

Methods:

Subjects

A cross-sectional study was performed on a sample of 53 single adolescents aged 16-17 years of normal weight and who had not suffered fetal distress at birth. All these adolescents were born at the Virgen de la Salud Hospital (Toledo, Spain), a regional hospital of 650 beds, placed in a town of approximately 80,000 inhabitants, giving medical support to 250,000 people. The volunteers, 120 families belonging to the area of study in Toledo, were contacted to participate in the study. Eighty adolescents, together with their respective parents, showed interest and attended the hospital where they were informed about the aims and details of the study. Written consent was requested and finally obtained in 53 volunteers before participation. All of them adequately filled in the nutritional survey, thus were included in the study. Reasons given for not participating were availability, attending classes, distance from the Hospital, type of analysis required, and concern about blood extraction. This study was approved by the Ethical Committee for Research Involving Human Subjects of the Hospital Virgen de la Salud de Toledo (Spain). The study protocol was performed in accordance with the ethical standards laid down in the 1961 Declaration of Helsinki (as revised in 2000).

Instruments and procedures:

Anthropometry. Height and weight were measured by standardized procedures. Weight was measured in underwear and without shoes with an electronic scale (Type SECA 861, Birmingham, UK) to the nearest 0.1 kg, and height was measured barefoot in the Frankfort horizontal plane with a telescopic height measuring instrument (Holtain® Ltd., Dyfed, UK) to the nearest 0.1 cm. Body mass index was calculated as body weight in kg divided by the square of height in meters. Fat body mass (in percentage) was assessed by bio impedance (Omron BF306, Hoofddorp, the Netherlands). Age and gender-specific cut points were used to assess their BMI category namely underweight, normal weight, overweight or obese[19]. The Conicity index[20] was calculated according to the following formula:

$$\text{Conicity Index} = \frac{\text{Waist Circumference (m)}}{0.109 \times \sqrt{\frac{\text{Body Weight (kg)}}{\text{Height (m)}}}}$$

Blood measurements. Blood samples were collected from the antecubital vein between 8:00 and 9:00 AM, after an overnight fast. Serum lipids were determined in a Hitachi Modular ISE® (Tokyo, Japan). Total cholesterol (TC) and triglycerides (TG) were tested according to standard enzymatic methods, while HDLc was determined by the Tietz direct method[21]. Low-density lipoprotein cholesterol (LDLc) was determined by direct method using cLDL-Plus in Hitachi 917 Roche Diagnostics® (Basel, Switzerland). Apolipoproteins (ApoA1 and ApoB) were determined by nephelometry

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(IMMAGE800 BECKMAN®, Mervue Galway, Ireland). Glucose was determined using the direct hexoquinase method of in Hitachi Modular ISE® (Tokyo, Japan). The LDLc/HDLc, CT/HDLc, ApoA1/ApoB, and the molar TG/HDLc ratios were calculated. Plasma leptin concentrations were determined by means of Sandwich ELISA (Human Leptin ELISA Kit, Abcam®100581), plasma adiponectin concentrations were determined by means of Sandwich ELISA (Human Adiponectin ELISA Kit, Abcam®99968), and plasma IGF1 concentrations were determined by means of Sandwich ELISA (Human IGF1 ELISA Kit, Abcam®100545).

Our laboratory participates in the Spanish Clinical Chemistry Society (SEQC) External Quality Evaluation Program, which follows UNE-EN-ISO 9001:2000 standards and is certified by AENOR. All assays were properly calibrated and performed under internal and external quality controls provided by the manufacturers and SEQC, respectively. Intra-assay and inter-assay variation coefficients were 0.8% and 1.7% for TC; 1.5% and 1.8% for TG; 0.9% and 1.85% for HDLc; 1% and 2.4% for ApoA1; 1.5% and 2.5% for ApoB; 1% and 1.7% for glucose, 1.5% and 4.9% for insulin, 4.4% and 6.6% for IGF1; 5.5% and 6.7% for leptin and 2.3% and 5.0% for adiponectin. The indices used to test insulin resistance or sensitivity was the Quantitative insulin sensitivity check index (QUICKI), calculated as 1/[log Insulin (mUI/L)+log Glucose (mg/dL)]. Homeostatic model assessment-insulin resistance (HOMA-IR), as: Glucose (mmol/L) x Insulin (μIU/mL)/22.5. HOMA-B evaluated the ability to produce insulin by beta-pancreatic cells under non-stimulation conditions according to the formula: HOMA-B= 20 x mUI/L glucose (mmol/L) - 3.5. HOMA-D was estimated to evaluate the relationship between basal glucose and basal insulin according to the formula: HOMA-D= HOMA-S x HOMA-B.

Dietary assessment. One month before the interview, a 72 hour dietary recall was sent to the families. On the interview day, volunteers delivered their recalls and any doubt about the records was solved. An interview for 1 to 1.5 hours with the adolescents together with their parents was performed. Food intake, food habits, and also cooking methods and location were registered. Participants completed a food frequency questionnaire (FFQ) that included several items classified according to food groups and based on the FFQs used and validated in the enKid study[(22)]. Photographs of sample portions were used to help estimate serving size and volume consumed. The dietitian conducting the survey reviewed, together with the volunteers and their parents, the usual frequency of consumption of each food (per day/week/month), together with the normal size of helping. The energy, nutrient intakes per person and the Healthy Eating Index (HEI) were calculated using a computer program (DIAL™ Madrid, Spain)[(23)] to evaluate diet quality consumed. The HEI index used, based on a 10-component, 100-point scale, is a slight modification for the Spanish population of that of Kennedy *et al.*[(24)] taking into account recommended energy intakes and the required servings. Diets with HEI-scores of ≤70 were labelled "inadequate", while those with HEI-scores of >70 were considered "adequate".

The Mediterranean Diet Adherence (MDA) score of 14 points used in the PREDIMED study was selected[(15,17)]. However, taking into account that wine should not be consumed by adolescents and information on alcoholic beverages in adolescence could be misleading, the MDA-score used was based on just 13 points. Each of the 13 components comprising the MDA-score contributing a maximum of one point to the final score.

Data analysis. The statistical analysis was performed with the SPSS® 15.0 software. Statistical significance was set up at $p < 0.05$. Descriptive statistics (median and interquartile range) and the U de Mann-Whitney test were used for statistical analysis to test gender differences on anthropometric, biochemical and dietary parameters. The relationships between HEI, MDA, anthropometrical and biochemical parameters were analyzed by the Spearman correlation test.

Results:

Table 1 summarizes the anthropometric and blood pressure results of the total male and female adolescents. Non-significant differences were found.

Table 1 Anthropometric parameters and blood pressure in adolescents of Area

	Total (n=53)	Boys (n=22)	Girls (n=31)	*P*
Weight (kg)	59.5 (16.5)	64.2 (20.0)	59.0 (13.5)	0.698
Height (cm)	166 (16.0)	164.7 (14.3)	166.5 (17.3)	0.698
Body mass index (kg/m^2)	21.5 (5.0)	21.8 (5.6)	21.3 (4.7)	0.652
Waist circumference (cm)	73.0 (14.0)	75.2 (14.9)	72.0 (13.0)	0.814
Hip circumference (cm)	95.0 (13.3)	97.0 (12.6)	92.0 (14.0)	0.426
Waist-to-hip ratio	0.76 (0.1)	0.76 (0.1)	0.76 (0.1)	0.993
Brachial perimeter (cm)	26.0 (4.7)	26.5 (5.0)	26.0 (5.0)	0.942
Tricipital fold (mm)	17.0 (12.0)	16.5 (11.5)	19.0 (12.0)	0.575
Body fat mass (%)*	21.2 (16.0)	20.0 (18.2)	23.4 (16.5)	0.718
Conicity index	1.11 (0.12)	1.13 (0.10)	1.10 (0.12)	0.248
Systolic blood pressure (mmHg)	116.0 (11.0)	115.0 (10.3)	118.0 (11.0)	0.170
Diastolic blood pressure (mmHg)	75.0 (10.5)	77.0 (10.3)	73.0 (10.0)	0.342

Results are reported as Median (interquartile range); U de Mann-Whitney (statistical significance was set up at $P < 0.05$). *Determined by bioimpedance.

DOI: 10.19230/jonnpr.2016.1.7.1087

Table 2 reportes results on glucose, insulin, insulin sensitivity/resistance markers, leptin and adiponectin in this selected population. No significant differences between males and females were observed.

Table 2 Glucose, insulin, and resistance/sensitivity insulin markers in adolescents of Area Toledo Study

	Total (n=53)	Boys (n=22)	Girls (n=31)	*P*
Glucose (mg/dL)	88.0 (11.5)	88.5 (10.0)	87.0 (13.0)	0.613
Insulin (mUI/L)	8.4 (5.5)	8.6 (4.0)	8.0 (8.0)	0.843
HOMA-IR	1.1 (0.7)	1.2 (0.5)	1.1 (1.0)	0.745
HOMA-B	107.5 (37.2)	105.5 (23.6)	116.0 (52.6)	0.957
HOMA-D	98.0 (40.9)	96.8 (39.6)	100.6 (48.3)	0.773
QUICKI	0.34 (0.04)	0.34 (0.03)	0.34 (0.05)	0.857
IGF1 (ng/ml)	304.0 (96.5)	291.5 (155.3)	305.0 (65.0)	0.878
Adiponectin (mg/mL)	26.0 (19.0)	25.9 (22.5)	26.0 (14.6)	0.935
Leptin (ng/mL)	6.3 (13.2)	8.2 (11.2)	6.1 (16.3)	0.671
Leptin/Adiponectin	0.26 (0.66)	0.30 (0.83)	0.25 (0.43)	0.448

Results are reported as Median (interquartile range); U de Mann-Whitney (statistical significance was set up at $P < 0.05$). HOMA, Homeostatic model assessment; QUICKI, Quantitative Insulin Sensitivity Check Index, marker of insulin sensitivity; IGF, Insulin-like growth factor.

Lipid and lipoprotein markers for adolescents are summarized in **Table 3**. Non-significant gender differences were found.

Table 3 Lipid and lipoprotein values in adolescents of Area Toledo Study

	Total (n=53)	Boys (n=22)	Girls (n=31)	*P*
TC (mg/dL)	151.0 (39.0)	155.5 (38.0)	149.0 (35.0)	0.705
HDLc (mg/dL)	57.0 (13.5)	58.0 (7.5)	57.0 (16.0)	0.658
LDLc (mg/dL)	96.0 (36.0)	95.5 (38.3)	96.0 (41.0)	0.626
TG (mg/dL)	60.0 (23.0)	65.0 (32.8)	58.0 (19.0)	0.271
ApoA1 (mg/dL)	141.0 (37.5)	143.0 (32.3)	139.0 (42.0)	0.814
ApoB (mg/dL)	74.2 (29.4)	67.6 (27.5)	75.6 (31.3)	0.620
TG/HDLc*	0.44 (0.24)	0.46 (0.23)	0.42 (0.20)	0.348
TC/HDLc +	2.51 (1.0)	2.58 (1.0)	2.43 (1.0)	0.658
LDLc/ HDLc	1.54 (0.80)	1.61 (0.62)	1.54 (0.83)	0.588
ApoA1/ApoB	2.05 (1.01)	2.10 (1.10)	1.88 (1.12)	0.732
HDLc/ApoA1	0.39 (0.09)	0.39 (0.10)	0.39 (0.10)	0.836
LDLc/ApoB	1.29 (0.31)	1.31 (0.25)	1.29 (0.31)	0.745

Results are reported as Median (interquartile range); U de Mann-Whitney (statistical significance was set up at $P < 0.05$). TC, total cholesterol; HDLc and LDLc, cholesterol transported by HDL and LDL, respectively; TG, triglycerides; Apo, apolipoprotein.*ratio molar;

Table 4 summarizes data for correlations founds between BMI and body fat mass (%) with selected anthropometric, biochemical values. Leptin appears significantly related ($p = 0.036$) to BMI in all adolescents, while to body fat mass in (all adolescents ($p < 0.001$) as well as in both genders (both $p < 0.001$)). Insulin, HOMA-IR and QUICKI appeared significantly related to BMI in all adolescents (at least $p = 0.035$) and girls (at least $p = 0.019$) while to body mass fat in all adolescents (at least $p = 0.002$). The conicity index was not significantly correlated with BMI or body fat mass. The conicity index highly correlated ($p < 0.001$) with waist circumference in male and female adolescents.

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Table 4. Spearmen correlations between BMI, Body Fat Mass (BMF) (%), conicity index and other anthropometrical and biochemistry parameters in all, boys and girls adolescents.

	BMI	BMF	TF	BP	WC	Insulin	HOMA-IR	QUICKI	Leptin	Leptin/adiponectin
BMI (All)		0.555	0.489	0.712	0.476	0.290	0.324	-0.329	0.288	
		P < 0.001	*P* < 0.001	*P* < 0.001	*P* < 0.001	*P* = 0.035	*P* = 0.018	*P* = 0.016	*P* = 0.036	
BMI (Boys)		0.467		0.649	0.466					
		P = 0.028		*P* < 0.001	*P* = 0.038					
BMI (Girls)		0.571	0.535	0.727	0.462	0.420	0.458	-0.461		
		P < 0.001	*P* = 0.002	*P* < 0.001	*P* = 0.009	*P* = 0.019	*P* = 0.010	*P* = 0.009		
		BMI	TF	BP	WC	Insulin	HOMA-IR	QUICKI	Leptin	Leptin/adiponectin
BFM (All)	0.555		0.800	0.498		0.408	0.403	-0.413	0.701	0.650
	P < 0.001		*P* < 0.001	*P* < 0.001		*P* = 0.002	*P* = 0.003	*P* = 0.003	*P* < 0.001	*P* < 0.001
BFM (Boys)	0.467		0.798						0.730	0.730
	P = 0.028		*P* < 0.001						*P* < 0.001	*P* < 0.001
BFM (Girls)	0.571		0.775	0.603					0.771	0.687
	P < 0.001		*P* < 0.001	*P* < 0.001					*P* < 0.001	*P* < 0.001
	BMI	BMF	TF	BP	WC	Insulin	HOMA-IR	QUICKI	Leptin	Leptin/adiponectin
CI (All)					0.734					
					P < 0.001					
CI (Boys)					0.809					
					P < 0.001					
CI (Girls)					0.689					
					P < 0.001					

Only significant correlations were shown. P
BMI, Body mass index; BMF, Body fat mass; BP, Brachial perimeter; CI, Conicity index; HOMA-IR, Homeostatic model assessment- Insulin resistance; QUICKI, Quantitative Insulin Sensitivity Check Index; TF, Tricipital fold; WC, waist circuference

Tables 5 and 6 report data of the diets consumed by the adolescents. Non-significant differences for the energy and nutrient intakes between males and females, except for polyunsaturated fatty acid (PUFA) that was marginally significantly higher in boys (p = 0.067), were found. Boys also displayed higher PUFA/SFA ratio (p = 0.002). Non-significant differences were observed between the diet quality (HEI and MDA) of boys and girls.

Table 5 Differences in energy, macronutrients, fiber and cholesterol among adolescents' diets of Area Toledo Study

	Total (n=53)	Boys (n=22)	Girls (n=31)	P
Energy intake (kcal)	2147 (392)	2279 (522)	2060 (259)	0.151
Energy intake (kJ)	8983 (1640)	9535 (2184)	8619 (1084)	0.151
Protein (g)	87.8 (26.0)	89.3 (20.0)	86.2 (33.0)	0.386
Carbohydrates (g)	238.6 (62.4)	244.5 (76.8)	235.1 (44.2)	0.608
Fats (g)	89.8 (28.0)	97.8 (37.0)	89.1 (17.0)	0.490
SFA (g)	30.0 (11.0)	30.0 (14.0)	29.8 (10.0)	0.401
MUFA (g)	41.0 (13.0)	46.8 (14.0)	40.6 (13.0)	0.520
PUFA (g)	9.9 (4.0)	11.4 (5.0)	9.5 (3.0)	0.067
w-6 PUFA/w-3 PUFA (g/g)	6.9 (2.9)	7.3 (3.1)	6.8 (2.3)	0.461
Fiber (g)	19.6 (9.0)	19.3 (13.0)	19.6 (8.0)	0.751
Cholesterol (mg)	354.5 (176.0)	392.0 (226.0)	349.0 (118.0)	0.601
Alcohol (g)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.835
Proteins (%kcal)	16.0 (4.0)	16.7 (3.0)	15.5 (5.0)	0.430
Carbohydrates (%kcal)	45.3 (6.0)	45.3 (6.0)	45.5 (7.0)	0.874
Fats (%kcal)	38.3 (6.0)	37.8 (8.0)	38.4 (6.0)	0.978
Alcohol (%kcal)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.835

Results are reported as Median (interquartile range); U de Mann-Whitney (statistical significance was set up at $P < 0.05$). SFA, Saturated fatty acids; MUFA, Monounsaturated fatty acids; PUFA, Polyunsaturated fatty acids.

Table 6 Differences in energy, macronutrients, fiber and cholesterol among adolescents' diets of Area Toledo Study

	Total (n=53)	Boys (n=22)	Girls (n=31)	*P*
SFA (%kcal)	13.0 (4.0)	13.0 (5.0)	12.9 (3.0)	1.000
MUFA (%kcal)	17.4 (4.0)	17.7 (3.0)	16.7 (4.0)	0.468
PUFA (%kcal)	4.3 (1.0)	4.7 (1.0)	4.2 (1.0)	0.062
SFA/Carbohydrates (g/g)	0.12 (0.26)	0.12 (0.26)	0.13 (0.20)	0.508
PUFA/SFA (g/g)	0.34 (0.0)	0.41 (0.0)	0.32 (0.0)	0.002
(MUFA+PUFA)/SFA (g/g)	1.7 (0.0)	1.8 (0.0)	1.6 (0.0)	0.130
Adherence to Mediterranean diet, score[a]	5.0 (2.0)	5.0 (2.0)	5.0 (2.0)	0.909
Healthy Eating Index (HEI), score[b]	64.9 (22.0)	64.0 (29.0)	67.0 (19.0)	0.859
HEI. Cereals-Legumes (0-10)	8.2 (2.4)	8.3 (2.9)	8.4 (2.39)	0.704
HEI. Vegetables (0-10)	6.6 (5.4)	7.3 (5.3)	6.0 (5.8)	0.281
HEI. Fruits (0-10)	7.6 (7.0)	7.4 (8.1)	8.4 (6.9)	0.496
HEI. Dairy (0-10)	6.5 (3.6)	6.8 (3.9)	6.5 (3.7)	0.765
HEI. Meat-Fish-Eggs (0-10)	10.0 (0.0)	10.0 (0.0)	10.0 (0.0)	0.818
HEI. Food variety (0-10)	5.0 (4.0)	5.5 (5.0)	5.0 (5.0)	0.419

Results are reported as Median (interquartile range); U de Mann-Whitney (statistical significance was set up at $P < 0.05$). SFA, Saturated fatty acids; MUFA, Monounsaturated fatty acids; PUFA, Polyunsaturated fatty acids. [a]Adherence to Mediterranean diet (0-13 score); [b]HEI, Health Eating Index (0-100 score).

JONNPR. 2016;1(7):244-253
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Figures 1 and 2 show the percentage of adolescents covering less than 66% of nutritional objectives and Recommended Dietary Intakes. A large percentage of boys and girls did not cover such percentages or has large macronutrient and micronutrient consumption.

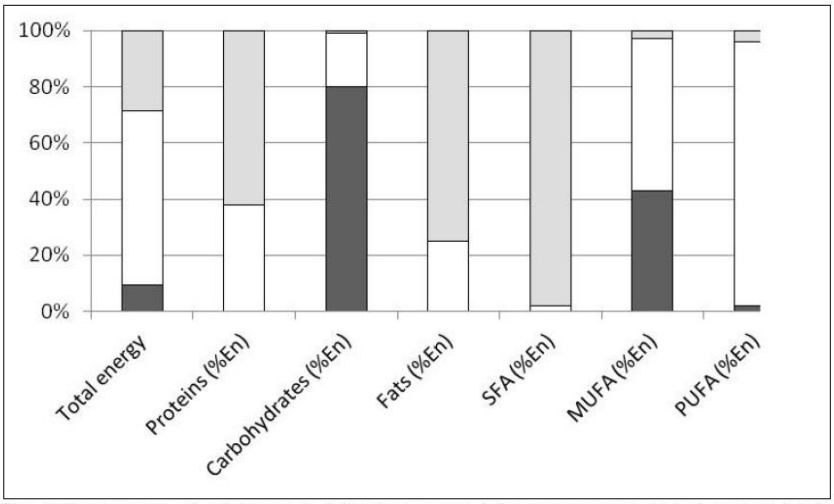

Fig. 1 Percentage of adolescents following or not following recommended energy intake (RDI) and nutritional objectives (NO) for macronutrient contribution to total energy according to [45,46]. ■ low intake (<70% RDI or <70 % NO); □ adequate intake (70-100% RDI or 70-100% NO), ■ high intake (>100% RDI or >100% NO).

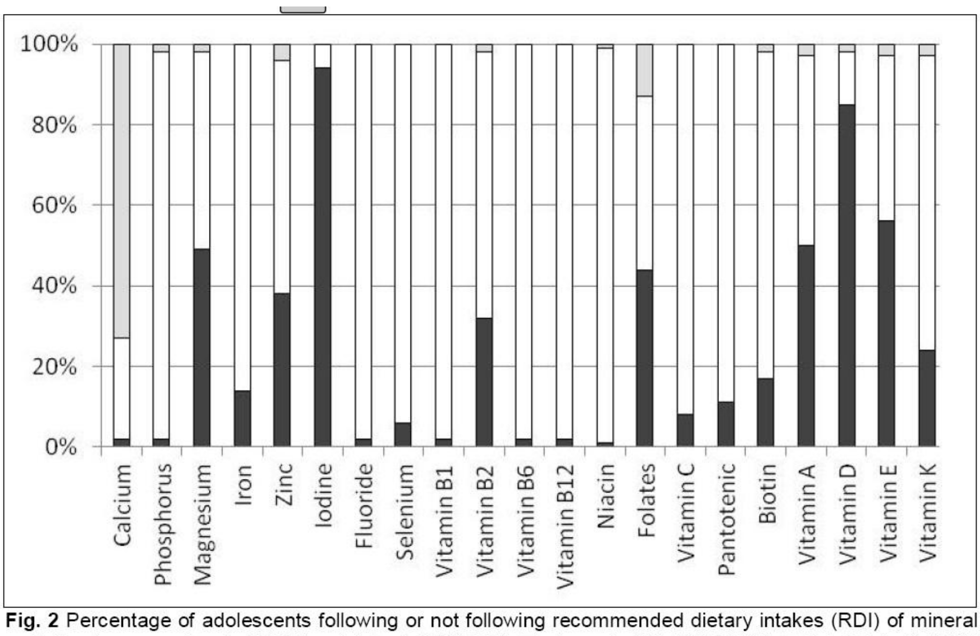

Fig. 2 Percentage of adolescents following or not following recommended dietary intakes (RDI) of mineral and vitamins according to [51] ■ low intake (<70% RDI); □ adequate (70-100% RDI), ■ high intake (>100% RDI).

Discussion:

Present results give extra data to the existing apparent paradox observed in some Spanish regions where inadequate diets co-exist with low CVD risk rates(4,6,25). Diet, anthropometry, lipoprotein, and insulin sensitivity markers were measured and correlated in an adolescent population belonging to one of the lowest CVD areas in Spain(4,6). Adolescents showed monotonous diets and moderate prevalence of being overweight but low prevalence of obesity. Diet quality measured by means of MDA or HEI did not significantly correlate with lipid-lipoprotein markers, but distinctly correlated with anthropometric or insulin resistance markers. Present results show that prevalence towards dyslipidemia and insulin resistance was not high, even when negative anthropometric and dietary profiles were present.

Anthropometric characteristics and blood pressure

Participants ranged in similar height to other adolescent Spanish groups(19). Contrasting with other European studies (26,27) where male adolescents are taller and heavier, no gender differences for BMI were observed. Being overweight is presently considered a cornerstone of CVD and metabolic syndrome(28,29). The prevalence of being overweight is increasing all over the world among children, adolescents, and adults(26-31). Twenty-six percent of the adolescents studied showed overweight but the obesity rate (1.9%) was lower than that observed in some Spanish Autonomic Communities or in the whole of Spain[29]. Moreno et al in the AVENA study[30] reported body fat percentage was highest in female adolescents of 16.5 years.

As already mentioned Spain still presents one of the lowest CVD rates in Europe[1], with the province of Toledo showing a medium-low cardiovascular mortality rate of 140 per 100.000 inhabitants[6]. Average systolic blood pressure tended to be lower while diastolic blood pressure was higher than that observed in the Helena Study[30]. The prevalence of high blood pressure (>120 mmHg and >80 mmHg for systolic and diastolic blood pressures respectively) was 11%. The similarity observed for blood pressure between boys and girls was observed in other studies for normal weight adolescents[27,31,32].

Serum cholesterol has increased, mainly in young populations, during the last few decades in Spain[25,33]. In fact, more than 20% of Spanish children have TC ≥5.14 mmol/l or ≥200 mg/dl[25]. The present adolescent population can be defined to be at low risk as 13% had TC ≥185 mg/dl; 18% LDLc ≥115mg/dl; 6% ≥110 mg/dl of TG and 19% HDLc <50mg/dL.

The molar TG/HDLc ratio has been used as a size and atherogenicity marker of LDL[34]. Volunteers display low atherogenicity values for LDL as the TG/HDLc ratio was much lower than 1.33, cut-off point suggested by Austin et al.[35].

The potential protective effect of estrogens on lipoprotein profile was poorly evident in the 16-17 year old adolescents in the Toledo Study, as lipid, lipoprotein, and Apo values, and CVD ratios were similar in both genders. Similarities in lipoprotein lipids and ratios could be related, in addition to the absence of anthropometrical characteristics, to the equivalence of diet composition as we will discuss below. Moreover, the low prevalence of obesity in both male and female adolescents also helps us to understand lipoprotein results due to the relationship between bodyweight and obesity and TG, and HDLc values[28,32].

Levels of glucose, insulin, HOMA-IR and QUICKI suggest adequate insulin sensitivity in present volunteers. Only 7.5% of adolescents present insulinemia >15 mUI/L, a value used as a cut-off point for hyperinsulinemia[36,37]. Twenty-five percent of adolescents showed HOMAS-IR > 1.5. However, considering that the mean and the 90th percentile values reported for HOMA-IR in Spanish children were 1.72 and 3.43[38], it can be deduced that a high percentage of our adolescent population showed high insulin sensitivity.

Normal leptinemia has been suggested for a leptin range of between 1 and 15 ng/mL[39]. Approximately 25% of adolescents showed leptinemia ≥15 mg/mL. However, according to the 3-30 μg/mL normality range for adiponectin[40], only 8% of adolescents were hypoadiponectinemics.

As for anthropometrical and lipoprotein parameters, non-significant gender differences were found for insulin resistance/sensitivity markers, adiponectin and leptin. Adiponectin has been described to play a protective role against insulin resistance. In fact, the low insulin resistance observed seems related to the normal adiponectin values found. Women show higher adiponectin and leptin values than men[40]. Thus the similarities observed in those hormones and in the leptin/adiponectin ratio suggest, again, the minor impact of estrogen at 16-17 years, at least in the present population studied.

It is interesting to point out the absence of significant relationships between BMI and body fat mass and lipid and lipoprotein markers. However, the conicity index showed significant correlation with TG and the TG/HDLc molar ratio. Ruperto et al[41] found that serum TG correlates with the conicity index but not with the fat mass. However, anthropometrical measurements, despite the relative normality of data obtained, appear significantly related to sensitivity/resistance markers. Thus, fat mass measured by bioimpedance correlated significantly with leptin and insulin/sensitivity markers in all adolescents. Leptinemia is highly dependent on fat mass and it is increased by being overweight and obese[42]. Leptin and BMI were not significantly related to boys and girls but weakly in all volunteers suggesting the subestimation of BMI in comparison to other anthropometric methods in the fat mass determination[20,43]. These results show the importance of measuring body fat mass instead of BMI.

General characteristics of adolescent diets

The diets of volunteers appear inadequate in several aspects and far from what is considered to be a prudent diet [5,44-46]. Dietary lipid profile was similar to the one observed in the present Spanish youth, where the energy contribution of saturated fatty acids (SFA) is about twice the 7% suggested as the secondary objective for optimum CVD prevention [22,46-48]. Nonetheless, the MUFA and PUFA energy contribution appears adequate. In addition, the contribution of PUFA ω-6 and ω-3 was inside the range suggested for an admissible intake according to the criteria of some scientific entities[45] that suggest that the PUFA ω-6 to PUFA ω-3 ratio have to be lower than 10, but preferably about 3-4. Nonetheless, FAO/WHO[46] has suggested that this ratio is not important when the contribution to total energy of both PUFA groups is in the recommended range. The absolute cholesterol intake was higher than that which was suggested in nutritional guidelines[45,46] while that of dietary fibre was lower according to the nutritional guidelines[5,11,45] but in line with that reported in the enKid[22] and AVENA[48] studies. The median for the PUFA ω-6/ PUFA ω-3 ratio in all, males and females was 6.9; 7.3 and 6.8, respectively and higher than the theoretical optimum mentioned previously. Paradoxically, the limitations of the study diet did not seem to negatively affect lipid and lipoprotein levels in the adolescents of the Toledo study, due to the lower prevalence of increased CVD risk factor observed.

HEI and DMA are two well accepted indices for assessing diet quality[15,23,49,50]. Adolescents displayed a MDA median of 5 over 13 points in all, males and females, clearly suggesting that the diet of volunteers was far from the Mediterranean diet profile. This dietary circumstance could be conditioned by the geographical situation and/or cultural and gastronomic traditions of Toledo, where the consumption of pork and pig products is rather high while that of fish is low. All this suggests the need to start information and formation programs in order to implement or recover the Mediterranean Diet as soon as possible. A large percentage of adolescents, displayed HEI lower than 70, with no differences between males and females. In addition 56.8 % of volunteers followed a diet that was unacceptable under both MDA and HEI points of

DOI: 10.19230/jonnpr.2016.1.7.1087

view. As HEI evaluates fat, SFA energy contribution, cholesterol intake, diet variety and the consumption of different food groups, it can be indicated that more than half of the adolescents were far from the nutritional objectives set[44].

MDA score significantly correlated with some anthropometrical data (p.e. fat mass, r =-0.359; *p* = 0.047; brachial perimeter, r=-0.416; *p* = 0.020; tricipital fold, r = -0.485; *p* = 0.006) in girls but not in boys. Negative significant correlation was found between MDA scores and tricipital fold values in all volunteers (r = -0.352; *p* = 0.011). On the other hand, HEI scores correlated significantly with insulin (r= -0.50; *p* = 0.021), HOMA-IR (r =-0.524; *p* = 0.015), and QUICKI (r = 0.604; *p* = 0.004) levels in boys but not in girls. HEI scores appear also correlated with insulin (r = -0.283; *p* = 0.042), HOMA-IR (r = -0.275; *p* = 0.049) and QUICKI (r = 0.314; *p* = 0.024) values in all adolescents. We are far from knowing the reasons implicated in the differences related to gender for these relationships obtained, as diet quality did not significantly differ between male and female adolescents. Gesteiro et al[50] have recently suggested that diet followed at pregnancy with low HEI or MDA scores are associated with high cord-blood insulin levels and insulin-resistance markers at birth in normal weight neonates.

Conclusions:

Present results clearly suggest the absence of gender effects in adolescent diet composition and quality. Despite adolescent diets being monotonous, and presenting low quality scores, the volunteers tested showed low prevalence of altered CVD risk factors and an acceptable level of insulin sensitivity. However, the 20% rates of being overweight and the correlations observed between HEI and insulin sensitivity/resistance markers demand urgent dietary education and intervention to decrease the negative effects of being overweight later in life and to recover traditional healthy diets.

Acknowledgements:

The study was granted by Projects AN/2006/22 and Pi2009/01 of the Fundación para la Investigación en Salud de Castilla La Mancha (FISCAM). Thanks are due to FISCAM for the contract of Tamara Guillen Casla.

FJSM is the Guarantor of the study and has collaborated in the design, data discussion, and writing of the paper. JMMS has participated in data acquisition, discussion, statistics and the writing of the paper. TGC has participated in the diet acquisition, SB has participated in the study design, data discussion and the writing of the paper, AAG in the study design and data acquisition. Thanks are due to Dr. Sagrario Perea and Dr. Alicia Ureta and to the Pharmacy, Biochemistry, and Pediatric services of the Hospital Virgen de la Salud of Toledo, Spain.

Conflicts of interest:

Authors declare that no conflicts of interest exist.

References

1. WHO (2011) Global status report on noncommunicable disease 2010. World Health Organization, Geneve (Switzerland)
2. Banegas JR, López-García E, Dallongeville J, Guallar E, Halcox JP, Borghi C, Massó-González EL, Jiménez FJ, Perk J, Steg PG, De Backer G, Rodríguez-Artalejo F (2011) Achievement of treatment goals for primary prevention of cardiovascular disease in clinical practice across Europe: the EURIKA study. *Eur Heart J* 32:2143-2152
3. Peña Quintana L (2003) Obesidad infantil y juvenil en España. Resultados del estudio enKid (1998-2000). *Med Clin (Barc)* 121:725-732
4. Royo-Bardonada MA, Gorgojo L, Martín-Moreno JM, Garcés C. Rodríguez-Artalejo F, Macías A, Mangas A, Rubio R, Barrio JL del, Oya M de (2003) Alimentación y nutrición en el Estudio Cuatro Provincias. In: Enfermedades cardiovasculares. Nutrición, genética y clínica. Garces C, Oya M de (eds.) Drug Farma S.L. Madrid, pp. 65-79
5. Varela-Moreiras G, Ruiz E, Valero T, Ávila JM, del Pozo S (2013) The Spanish diet: an update. *Nutr Hosp* 28 suppl. 5:13-20
6. Rodríguez Artalejo F, Banegas JR (2003) Las variaciones geográficas en las enfermedades cardiovasculares en España. En: Garcés C, Oya M de (eds.). Enfermedades cardiovasculares. Nutrición, genética y clínica. Drug Farma SL & Esteve, Madrid, pp. 11-23
7. Santaliestra-Pasías AM, Mouratidou T, Huybrechts I, Beghin L, Cuenca-García M, Castillo MJ, Galfo M, Hallstrom L, Kafatos A, Manios Y, Marcos A, Molnar D, Plada M, Pedrero-Chamizo R, Widhalm K, De Bourdeaudhuij I, Moreno LA (2014) Increased sedentary behaviour is associated with unhealthy dietary patterns in European adolescents participating in the HELENA study. *Eur J Clin Nutr* 68:300-308
8. Serra-Majem L, García-Closas R, Ribas L, Pérez-Rodrigo C, Aranceta J (2001) Food patterns of Spanish school children and adolescents: The enKid Study. *Public Health Nutr* 4: 1433-1438
9. Gesteiro E. Bastida S, Rodríguez-Bernal B, Sánchez-Muniz FJ (2015). Adherence to Mediterranean diet during pregnancy and serum lipid, lipoprotein and homocysteine concentrations at birth. *Eur J Nutr* 54:1191-1199.
10. Moreiras O, Carbajal A, Cabrera L, Cuadrado C (2009) Ingestas recomendadas para la población española. In Tablas de composición de alimentos. 13th Ed. Ediciones Pirámide. Madrid

DOI: 10.19230/jonnpr.2016.1.7.1087

11. Institute of Medicine, Food and Nutrition Board (2006) Dietary reference intakes: The essential guide of nutrient requirements. National Academic Press. Washington DC
12. Trichopoulou A, Bamia C, Trichopoulos D (2005) Mediterranean diet and survival among patients with coronary heart disease in Greece. *Arch Intern Med* 65:929-935
13. Estruch R, Ros E, Salas-Salvadó J, Covas MI, Corella D, Arós F, Gómez-Gracia E, Ruiz-Gutiérrez V, Fiol M, Lapetra J, Lamuela-Raventos RM, Serra-Majem L, Pintó X, Basora J, Muñoz MA, Sorlí JV, Martínez JA, Martínez-González MA, for the PREDIMED Study Investigators (2013) Primary prevention of cardiovascular disease with a Mediterranean diet. *N Engl J Med* 368:1279-1290
14. Sánchez-Muniz FJ (2007) Aceite de oliva, clave de vida en la Cuenca Mediterránea. *An R Ac Nac Far* 73:653-692
15. Estruch R, Martínez-González MA, Corella D, Salas-Salvadó J, Ruiz-Gutiérrez V, Covas MI, Fiol M, Gómez-Gracia E, López-Sabater MC, Vinyoles E, Arós F, Conde M, Lahoz C, Lapetra J, Sáez G, Ros E; PREDIMED Study Investigators (2006) Effects of a Mediterranean-style diet on cardiovascular risk factors: a randomized trial. *Ann Intern Med* 145:1–11
16. da Silva R, Bach-Faig A, Raidó Quintana B, Buckland G, Vaz de Almeida MD, Serra-Majem L (2009) Worldwide variation of adherence to the Mediterranean diet, in 1961-1965 and 2000-2003. *Public Health Nutr12*(9A):1676-1684
17. Salas-Salvadó J, Bulló M, Babio N, Martínez-González MÁ, Ibarrola-Jurado N, Basora J, Estruch R, Covas MI, Corella D, Arós F, Ruiz-Gutiérrez V, Ros E; PREDIMED Study Investigators (2011) Reduction in the incidence of type 2 diabetes with the Mediterranean diet: results of the PREDIMED-Reus nutrition intervention randomized trial. *Diabetes Care* 34:14-19
18. Buckland G, Bach-Faig A, Serra-Majem L (2008) Obesity and the Mediterranean diet: a systematic review of observational and intervention studies. *Obes Rev*; 9:582-593
19. Hernandez M, Castellet J, Narvaiza JL, Rincon I, Ruiz E, Sánchez B, Sobradillo B, Zurimendi A (1988) Curvas y tablas de crecimiento. Instituto sobre Crecimiento y Desarrollo. Fundacion F. Orbegozo, Madrid
20. Valdez R, Seidell JC, Ahn YI, Weiss KM. (1993) A new index of abdominal adiposity as an indicator of risk for cardiovascular disease. A cross population study. *Int J Obes Relat Metab Disord* 17:77–82
21. Tietz NW (1991) Clinical Guide to laboratory tests, 2nd edition. Saunders Co, New York, USA.
22. Serra-Majem L, García-Closas R, Ribas L, Pérez-Rodrigo C, Aranceta J (2001) Food patterns of Spanish schoolchildren and adolescents: The enKid Study. *Public Health Nutr* 4:1433-1438
23. Ortega RM, López-Sobaler AM, Andrés P, Requejo AM, Aparicio A, Molinero LM (2004) DIAL software for assessing diets and food calculations. Departamento de Nutrición (UCM) and Alce Ingeniería SA Madrid. http://www.alceingenieria.net/nutricion.htm.Accessed October 2012
24. Kennedy ET, Ohls J, Carlson S, Fleming K (1995) The Healthy Eating Index: design and applications. *J Am Diet Assoc*; 95:1103-1108
25. Garcés C, de Oya M. (2005) Niños y lípidos. *Clin Invest Arterioscl* 17:176-182
26. Sesé MA, Jiménez-Pavón D, Gilbert CC, González-Gross M, Gottrand F, de Henauw S, Breidenassel C, Wärnberg J, Widhalm K, Molnar D, Manios Y, Cuenca-García M, Kafatos A, Moreno LA; HELENA Study Group. (2012) Eating behaviour, insulin resistance and cluster of metabolic risk factors in European adolescents. The HELENA study. *Appetite* 59:140-147
27. Schwandt P, Scholze JE, Bertsch T, Liepold E, Haas GM. (2015) Blood pressure percentiles in 22,051 German children and adolescents: The PEP Family Heart study. *J Hypertens* 28: 672-679.
28. Daniels SR, Arnett DK, Eckel RH, Gidding SS, Hayman LL, Kumanyika S, Robinson TN, Scott BJ, St Jeor S, Williams CL (2005) Overweight in children and adolescents: pathophysiology, consequences, prevention, and treatment. *Circulation* 111:1999-2012
29. Varela-Moreiras G, Alguacil Merino LF, Alonso Aperte E, Aranceta Bartrina J, Avila Torres JM, Aznar Laín S, et al. (2013) CONSENSUS DOCUMENT AND CONCLUSIONS - Obesity and sedentarism in the 21st century: what can be done and what must be done? *Nutr Hosp* 28, Suppl 5:1-12
30. Moreno LA, Mesana MI, Fleta J, Ruiz JR, González-Gross M, Sarría A, Marcos A, Bueno M; AVENA Study Group (2005) Overweight, obesity and body fat composition in Spanish adolescents. The AVENA Study. *Ann Nutr Metab* 49:71-76
31. Stoner L1, Faulkner J, Westrupp N, Lambrick D (2015) Sexual differences in central arterial wave reflection are evident in prepubescent children. *J Hypertens.* 33: 304-307.
32. López Elcarte R, Villa Elizaga I, Sada Goñi J, Gasco Eguiluz M, Oyarzábal Irigoyen M, Sola Mateos A, Martínez González A, Elcarte López T, Ayensa Mezquiriz I, Castiella Lafuente F (1993) Prevalence of arterial hypertension, hyperlipidemia and obesity in the infant-child population of Navarra. Association of risk factors. *An Esp Pediatr* 38:130-137
33. Plaza Pérez I, Villar Alvarez F, Mata López P, Pérez Jiménez F, Maiquez Galán A, Casasnovas Lenguas JA, Banegas Banegas JR, Tomás Abadal L, Rodríguez Artalejo F, Gil López E (2000) Control of cholesterolemia in Spain, 2000. A tool for cardiovascular prevention. *Rev Esp Cardiol* 53:815-837
34. Boizel R, Benhamou PY, Lardy B, Laporte F, Foulon T, Halimi S (2000) Ratio of triglycerides to HDL cholesterol is an indicator of LDL particle size in patients with type 2 diabetes and normal HDL cholesterol levels. *Diabetes Care* 23:1679-1685

35. Austin MA, King MC, Vranizan KM, Krauss R (1990) Atherogenic lipoprotein phenotype. A proposed genetic marker for coronary heart disease risk. *Circulation* 82:495-506
36. Ten S, MacLaren N (2004) Insulin resistance syndrome in children. *J Clin Endocrinol Metab* 89:2526-2539
37. Gesteiro E, Bastida S, Sánchez-Muniz FJ (2009) Insulin resistance markers in term, normoweight neonates. The Mérida cohort. *Eur J Pediatr* 168: 281-288
38. García Cuartero B1, García Lacalle C, Jiménez Lobo C, González Vergaz A, Calvo Rey C, Alcázar Villar MJ, Díaz Martínez E (2007) The HOMA and QUICKI indexes, and insulin and C-peptide levels in healthy children. Cut off points to identify metabolic syndrome in healthy children. *An Pediatr (Barc)* 66:481-490
39. Sinha MK, Opentanova I, Ohannesian JP, Kolaczynski JW, Heiman ML, Hale J, Becker GW, Bowsher RR, Stephens TW, Caro JF (1996) Evidence of free and bound leptin in human circulation. Studies in lean and obese subjects and during short-term fasting. *J Clin Invest* 98:1277-1282
40. Arita Y, Kihara S, Ouchi N, Maeda K, Kuriyama H, Okamoto Y, Kumada M, Hotta K, Nishida M, Takahashi M, Nakamura T, Shimomura I, Muraguchi M, Ohmoto Y, Funahashi T, Matsuzawa Y (2002) Adipocyte-derived plasma protein adiponectin acts as a platelet-derived growth factor-BB-binding protein and regulates growth factor induced common postreceptor signal in vascular smooth muscle cell. *Circulation* 105:2893-2898
41. Ruperto M, Barril G, Sánchez-Muniz FJ (2013) Conicity index as a contributor marker of inflammation in haemodialysis patients. *Nutr Hosp* 28, 1740-1747
42. Mendelson M1, Michallet AS, Monneret D, Perrin C, Estève F, Lombard PR, Faure P, Lévy P, Favre-Juvin A, Pépin JL, Wuyam B, Flore P (2015) Impact of exercise training without caloric restriction on inflammation, insulin resistance and visceral fat mass in obese adolescents. *Pediatr Obes.* 10:311-319.
43. Minghelli B, Nunes C, Oliveira R (2013) Prevalence of overweight and obesity in portuguese adolescents: comparison of different anthropometric methods. *N Am J Med Sci* 5:653-659
44. Agencia Española de Seguridad Alimentaria y Nutrición (AESAN) y Fundación Española de la Nutrición (FEN) (ed.). (2013) Libro Blanco de la Nutrición en España. (Varela Mosquera, G general coordinator; Requejo Marcos AM, Ortega Anta RM, module coordinators). Agencia Española de Seguridad Alimentaria y Nutrición (AESAN) y Fundación Española de la Nutrición (FEN). ISBN 978-84-938865-2-3
45. SENC, Sociedad Española de Nutrición Comunitaria (2004) La Guía de la Alimentación Saludable Nueva Pirámide de la Alimentación (SENC 2004). En: www.portalesmedicos.com/noticias/guia_alimentacion.htm, www.ucm.es/info/nutri1/.../PiramideRecomendadaSENC 2004
46. FAO/WHO (2010) The Joint FAO/WHO expert consultation on fats and fatty acids in human nutrition. FAO food and nutrition paper 91. Food and Agriculture Organization of the United Nations, Rome
47. González-Gross M, Castillo MJ, Moreno L, Nova E, González-Lamuño D, Pérez-Llamas F, Gutiérrez A, Garaulet M, Joyanes M, Leiva A, Marcos A (2003) Alimentación y valoración del estado nutricional de los adolescentes españoles (Proyecto AVENA). Evaluación de riesgos y propuesta de intervención I. Descripción metodológica del estudio. *Nutr Hosp* 18: 15-28
48. Wärnberg J, Ruiz JR, Ortega FB, Romeo J, Gónzalez-Gross M, Moreno LA, García-Fuentes M, Gómez S, Nova E, Díaz LE, Marcos A y grupo AVENA (2006) Estudio AVENA* (Alimentación y valoración del estado nutricional en adolescentes). Resultados obtenidos 2003-2006. *Pediatr Integral* Suppl (1):50-55
49. Koning L, Chiuve SE, Fung TT, Willett WC, Rimm EB, Hu FB (2011) Diet-quality scores and the risk of type 2 diabetes in men. *Diabetes Care* 34:1150-1156
50. Gesteiro, E; Rodríguez Bernal, B; Bastida, S., Sánchez-Muniz, FJ (2012) Maternal diets with low healthy eating index or mediterranean diet adherence scores are associated with high cord-blood insulin levels and insulin resistance markers at birth. *Eur J Clin Nutr*; 66:1008-1015
51. Departamento de Nutrición (2008) Recommended dietary intakes of energy and nutrients for the Spanish population (Ingestas recomendadas de energía y nutrientes para la población Española (reviewed in 2008). In: Food Composition Tables (Tablas de Composición de Alimentos). Moreiras O, Carbajal A, Cabrera L, Cuadrado C (eds.). Pirámide. Madrid