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Original article

Relation between plasma antioxidant vitamin levels, adiposity and cardio-metabolic profile in adolescents: Effects of a multidisciplinary obesity programme

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SUMMARY

Background & aims: In vivo and in vitro evidence suggests that antioxidant vitamins and carotenoids may be key factors in the treatment and prevention of obesity and obesity-associated disorders. Hence, the objective of the present study was to determine the relationship between plasma lipid-soluble antioxidant vitamin and carotenoid levels and adiposity and cardio-metabolic risk markers in overweight and obese adolescents participating in a multidisciplinary weight loss programme.

Methods: A therapeutic programme was conducted with 103 adolescents aged 12–17 years old and diagnosed with overweight or obesity. Plasma concentrations of α -tocopherol, retinol, β -carotene and lycopene, anthropometric indicators of general and central adiposity, blood pressure and biochemical parameters were analysed at baseline and at 2 and 6 months of treatment.

Results: Lipid-corrected retinol ($P < 0.05$), β -carotene ($P = 0.001$) and α -tocopherol ($P < 0.001$) plasma levels increased significantly, whereas lipid-corrected lycopene levels remained unaltered during the treatment. Anthropometric indicators of adiposity ($P < 0.001$), blood pressure ($P < 0.01$) and biochemical parameters ($P < 0.05$) decreased significantly, whereas fat free mass increased significantly ($P < 0.001$). These clinical and biochemical improvements were related to changes in plasma lipid-corrected antioxidant vitamin and carotenoid levels. The adolescents who experienced the greatest weight loss also showed the largest decrease in anthropometric indicators of adiposity and biochemical parameters and the highest increase in fat free mass. Weight loss in these adolescents was related to an increase in plasma levels of lipid-corrected α -tocopherol ($P = 0.001$), β -carotene ($P = 0.034$) and lycopene ($P = 0.019$).

Abbreviations: ApoA1, apolipoprotein A1; ApoB, apolipoprotein B; BMI, body mass index; CRP, C-reactive protein; CVD, cardiovascular diseases; DBP, diastolic blood pressure; FFM, fat free mass; FMI, fat mass index; HDL, high density lipoprotein; LC, lipid-corrected; LDL, low density lipoprotein; SDS-BMI, BMI standard deviation score; SBP, systolic blood pressure; TAG, triacylglycerols; UHPLC, Ultra High Performance Liquid Chromatography.

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Conclusions: Plasma lipid-soluble antioxidant vitamin and carotenoid levels are associated with reduced adiposity, greater weight loss and an improved cardio-metabolic profile in overweight and obese adolescents.

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1. Introduction

The prevalence of obesity has increased in recent decades and this disorder is now considered a 21st century epidemic. It is the most common nutritional issue among children and adolescents in developed countries, but has also reached alarming values in developing countries [1].

Obesity is an important risk factor of cardiovascular diseases (CVD) and is strongly related to other cardiovascular risk factors such as hypertension, diabetes, dyslipidemia and inflammation [2], therefore onset of obesity is of particular concern in children and adolescents [3,4]. It has been found that similar to obese adults, obese children present a higher degree of oxidative stress than their normal weight counterparts [5]. Moreover, obesity is linked to decreased plasma antioxidant vitamin levels and antioxidant capacity [6]. Thus, the scientific evidence suggests that antioxidant vitamins and carotenoids may be key factors in the treatment and prevention of obesity and obesity-associated disorders.

Recent studies have indicated that vitamin A [7] and β -carotene [8] are important regulators of body fat reserves. As an example, retinoic acid derived from vitamin A and β -carotene is involved in the expression of lipogenic transcription factors [7,8]. Furthermore, it has been observed that vitamin A and carotenoids present anti-inflammatory properties [9–11] and have an antioxidant action [10–12]. Within the carotenoids, lycopene has the highest antioxidant capacity compared to α -tocopherol and β -carotene [13]. Numerous epidemiological studies have shown a significant inverse association between plasma or tissue lycopene levels and the incidence of CVD or CVD risk factors (atherosclerosis, hypertension, diabetes, metabolic syndrome, inflammation and oxidative stress) [12]. Also, it has been suggested that lycopene can prevent inflammation [11] by decreasing transcription factor activation and secretion of inflammatory markers in adipose tissue [9].

Vitamin E is another lipid-soluble vitamin that presents a high antioxidant capacity [13,14]. Recently, it was found that vitamin E is involved in the expression of genes associated with glucose and lipid metabolism [15,16]. In addition, some authors have observed a lower incidence of chronic disease and cardiovascular events when dietary intake of vitamin E is increased [14].

The study of changes in anthropometric and metabolic parameters and plasma antioxidant levels in obese children is useful in order to determine their associations and establish dietary regimes aimed at reducing the prevalence of paediatric obesity and associated pathologies. Nonetheless, to our knowledge, few studies have focussed in this issue. Hence, the objective of the present study was to determine the relationship between plasma levels of α -tocopherol, retinol, β -carotene and lycopene and adiposity and cardio-metabolic risk markers in overweight and obese adolescents participating in a multidisciplinary weight loss programme.

2. Materials and methods

2.1. Ethics statement

This study was conducted in accordance with the ethical rules of the Helsinki Declaration (Hong Kong revision, September 1989,

Edinburgh revision 2000 and Korea revision 2008), the European Economic Community (EEC) Good Clinical Practice guidelines (document 111/3976/88 of July 1990) and current Spanish law, which regulates clinical research on humans (Royal Decree 561/1993 regarding clinical trials). Written informed consent was obtained from all adolescents and their parents, and the study was approved by the local ethics committees. Data obtained during the intervention was confidential and restricted to the participating investigators.

2.2. Participants and study design

The study sample used in the present analysis comprised 103 adolescents aged 12–17 years old and diagnosed with overweight and obesity at four hospitals located in different Spanish cities (Granada, Madrid, Pamplona and Zaragoza). The inclusion criteria were as follows: to be overweight or obese as defined by International Obesity Task Force age- and sex-specific body mass index (BMI) values [17], to be Spanish or to have been educated in Spain, and to be free of any other diagnosed disease. Adolescents receiving pharmacological treatment or diagnosed with anorexia, bulimia or any other eating disorder except binge eating disorder, were excluded. All selected adolescents were treated as part of the EVASYON Study (Development, implementation and evaluation of the efficacy of a therapeutic programme for adolescents with overweight and obesity: comprehensive education on nutrition and physical activity) [18].

The EVASYON project was an intervention study in a cohort of overweight and obese adolescents. It comprised a long-term (approximately 13 months) multidisciplinary treatment programme based on a calorie-restricted diet (10–40%), increased physical activity (at least 60 min/day, 5 days a week), psychological therapy and nutritional education. The maximum energy intake was 1800 kcal/day for females and 2200 kcal/day for males. Macronutrient distribution was 50% of energy from carbohydrates, 30% from fat and 20% from proteins. The adolescents were treated in groups of a maximum of 10 subjects and each group had 20 visits during the intervention. The treatment comprehended two stages, an intensive intervention phase (1st to 9th visits) with weekly checks during the first 2 months, and an extensive intervention phase (10th to 20th visits) where adolescents were monitored monthly until the end of programme. The complete and detailed methodology of the EVASYON Study has been described elsewhere [18].

2.3. Dietary intake and physical condition

Dietary intake was assessed by applying 72-h dietary record and a semi-quantitative food-frequency questionnaire, previously validated. Data of food intake by 72-h dietary record were transformed into food volume/weight (in mL or g). The nutrient consumption was determined using the latest available information in food-composition tables from Spain. Vitamins and carotenoids were estimated from the intake of respective food sources.

To evaluate the physical activity, several tests and questionnaire were used, such as Physical Activity Questionnaire for Adolescents

(PAQ-A), Course navette or 20-m Shuttle run test, Handgrip strength, 4 × 10-m shuttle-run, among others. The complete and detailed methodology has been described elsewhere [18].

2.4. Anthropometry, pubertal development and resting blood pressure

Body weight (kg) was measured without shoes and with light clothing to the nearest 0.05 kg using a standard beam balance. Triceps, biceps, subscapular, suprailiac, thigh and calf skin-fold thicknesses were measured on the left side of the body to the nearest 0.2 mm using a Holtain skin-fold calliper. All the anthropometric variables were measured consecutively in triplicate and averaged. For all the anthropometric measurements, intra-observer reliability was >95% and inter-observer reliability was >90%.

Pubertal development was assessed in accordance with the 5-stage system established by Tanner [19]. Each stage describes pubic hair and breast development in girls and pubic hair and genital development in boys.

Blood pressure was measured using a validated digital automatic blood pressure monitor (Omron M6, Omron Health Care Co., Ltd., Kyoto, Japan) in accordance with the International Protocol of the European Society of Hypertension [20].

2.5. Biochemical and metabolic analysis

Blood was collected by venipuncture after an overnight fast. It was then centrifuged and aliquots of plasma or serum were stored at -80°C until analysis.

Total cholesterol, high density lipoprotein (HDL) cholesterol, triacylglycerols (TAG) and glucose were analysed using an Olympus AU2700 biochemical autoanalyser (Olympus, Melville, NY, USA). The coefficients of variance obtained were 2% for total cholesterol, 2% for HDL cholesterol and 3% for TAG. Very-low-density lipoprotein (VLDL) cholesterol and low density lipoprotein (LDL) cholesterol were calculated from existing values for cholesterol, HDL cholesterol and triglycerides [21]. Apolipoprotein B (apoB) and A1 (apoA1), insulin and C-reactive protein (CRP) were analysed using the methodology described in full detail elsewhere [18].

2.6. Determination of plasma antioxidant vitamins and carotenoids

Plasma levels of α -tocopherol, retinol, β -carotene and lycopene were determined at three different periods: before starting treatment, at 2 months and at 6 months of intervention. Analyses were carried out by Ultra High Performance Liquid Chromatography (UHPLC). Individual stock standard solutions were prepared for α -tocopherol, α -tocopherol acetate, retinol and retinyl acetate in ethanol, while β -carotene and lycopene standard solutions were prepared in dichloromethane:methanol (2:1). Concentration ranges for calibration were 5–150 $\mu\text{g}/\text{mL}$ (α -tocopherol) and 0.1–5 $\mu\text{g}/\text{mL}$ (retinol, β -carotene, lycopene). Sample analysis was performed as follows: 300 μL of previously vortexed plasma was de-proteinated with 500 μL of internal standard mixture (0.6 $\mu\text{g}/\text{mL}$ retinyl acetate and 17 $\mu\text{g}/\text{mL}$ α -tocopherol acetate in ethanol), vortexed for 1 min and extracted by centrifugation (4000 rpm, 5 min, 21 – 22°C) twice with 1 mL of hexane. The organic phase obtained was filtered using Acrodisc 13 mm Syringe Filter equipped with 0.2 μm GHP membranes, and was then evaporated to dryness under nitrogen atmosphere at 35°C . The residues were reconstituted to 50 μL with a solution of dichloromethane:methanol (2:1) and vortexed for 15 s. Chromatographic determination was performed using an Acquity Ultra Performance Liquid Chromatographic System (UPLC) (Waters, Milford, MA, USA) equipped with a binary solvent delivery module and a 2996 Photodiode Array (PDA)

detector. An Acquity UPLC BEH C18 column (2.1 mm i.d. × 50 mm, 1.7 μm particle size) (Waters, Milford, MA, USA) and an Acquity UPLC BEH C18 Van Guard Pre-column (2.1 mm i.d. × 5 mm, 1.7 μm particle size) (Waters, Milford, MA, USA) were used. Column and autosampler temperatures were 30°C and 10°C , respectively. Methanol was used as mobile phase at a flow rate of 0.6 mL/min. Injection volume was 3 μL and total run time 3 min. The wavelengths selected were 325, 292 and 450 nm for retinol, tocopherol and carotenoid analysis, respectively. The validity of the UHPLC method was assessed by including serum calibration standards and bi-level serum controls (Chromsystems, Munich, Germany). Method validation results are presented in Table 1.

2.7. Statistical analysis

Results are presented as means \pm standard deviation (SD) or standard error of the mean (SEM). SPSS 20.0 (SPSS Inc, Chicago, IL, USA) was used for statistical analyses. Changes in clinical or biochemical parameters and plasma lipid-soluble antioxidant vitamin and carotenoid levels were analysed by general linear models using Bonferroni post hoc correction. To evaluate the association between changes in antioxidant vitamins and carotenoids and anthropometric indicators of adiposity, blood pressure and biochemical parameters at 2 and 6 months of intervention, linear regression models were applied, adjusted for age, sex, body mass index standard deviation score (SDS-BMI) and Tanner stages at baseline, changes in intake of energy and corresponding vitamins or carotenoids at 6 months respect to baseline, and degree of physical activity. General linear models were also carried out to examine the differences between changes in clinical and biochemical parameters and changes in plasma vitamin and carotenoid levels at 6 months of intervention in the different weight loss groups, controlling for potential confounding factors (sex, age, SDS-BMI, Tanner stage and the corresponding variable at baseline, changes in intake of energy and corresponding vitamins or carotenoids at 6 months, and degree of physical activity). To determine whether changes in parameters studied were significant in each group of weight loss, estimated marginal means were used. For all analyses, two-sided significance was determined at a $P < 0.05$.

3. Results

The clinical and biochemical characteristics, physical condition and dietary intake of adolescents, at baseline and at 2 and 6 months of treatment, are given in Table 2. Participants' mean age and baseline body mass index (BMI) were 14.09 ± 1.21 years old and 31.4 ± 0.49 kg/m^2 , respectively; 46% were males and 54% females. It was found that weight and anthropometric indicators of total adiposity [BMI, SDS-BMI, body fat and fat mass index (FMI)] and central adiposity (waist circumference) decreased significantly during the 6 months of intervention ($P < 0.001$), whereas the percentage of fat free mass (FFM) increased ($P < 0.001$). Systolic (SBP) and diastolic blood pressure (DBP) improved after 2 months of treatment ($P = 0.002$ and <0.001 , respectively), subsequently remaining unchanged until the end of treatment. Most of the biochemical parameters (glucose, insulin, cholesterol, HDL cholesterol, LDL cholesterol, apoA1, apoB and CRP) decreased during the intensive intervention phase ($P < 0.05$). However, the TAG and apolipoprotein B/apolipoprotein A1 (apoB/apoA1) ratio only changed at the end of the programme ($P = 0.001$), showing values lower than those measured at baseline and 2 months of intensive treatment. During the extensive intervention phase, some of the parameters which had decreased during the intensive period, such as cholesterol, HDL cholesterol and apoA1, became higher with

Table 1
Parameters of UHPLC method validation for lipid-soluble vitamins and carotenoids.

Analytes	Repeatability (n = 10)		Reproducibility (n = 20)		Linearity		LOD (ng/mL)	LOQ (ng/mL)	Recovery (%)
	Means ± SD (µg/mL)	CV (%)	Means ± SD (µg/mL)	CV (%)	Equation	r ²			
α-Tocopherol	18.87 ± 0.20	1.04	18.98 ± 0.36	1.89	y = 0.0196x + 0.0166	0.9999	29	99	103
Retinol	0.62 ± 0.01	1.89	0.61 ± 0.03	4.28	y = 0.2559x – 0.0105	1	1.6	5.2	97
Lycopene	0.15 ± 0.01	4.22	0.15 ± 0.01	6.96	y = 0.4508x + 0.006	0.9998	2.2	7.3	92
β-Carotene	0.09 ± 0.00	4.62	0.08 ± 0.01	6.12	y = 0.5581x + 0.007	0.9999	1.8	6.1	91

CV, coefficient of variation; LOD, limit of detection; LOQ, limit of quantification.

respect to 2 months ($P < 0.001$), but did not return to the baseline values. Regarding to physical condition, it was observed that agility and cardiorespiratory endurance improved from 2 months ($P < 0.001$), whilst handgrip strength increased significantly at the end of intervention ($P < 0.001$). On the other hand, the intake of energy, carbohydrates, proteins, lipids, vitamin A and vitamin E decreased ($P < 0.001$), whereas β-carotene intake enhanced ($P = 0.014$) and lycopene tended to increase ($P = 0.064$).

Changes in plasma α-tocopherol, retinol, lycopene and β-carotene levels during treatment are presented in Fig. 1. Values are expressed as concentration, and also applying a lipid correction [6] by dividing the concentration by the sum of cholesterol and TAG. It was observed that lipid-corrected (LC) retinol increased significantly at 2 months of treatment ($P < 0.05$), whereas LC β-carotene ($P = 0.001$) and LC α-tocopherol ($P < 0.001$) were significantly

higher in the final stage of the treatment. LC lycopene levels did not change during the intervention.

Table 3 shows the associations between the changes in plasma antioxidant vitamin and carotenoid levels and the changes in several clinical and biochemical parameters at 2 and 6 months, in relation to baseline values. Since LC lycopene remained unaltered during the treatment, its correlations with other parameters have not been explored. As shown in Fig. 1, the changes LC retinol levels were significant at 2 months of treatment. Thus, the increase in LC retinol was associated with a reduction in cholesterol and TAG ($P < 0.001$).

The changes in LC α-tocopherol and LC β-carotene levels were significant at 6 months of intervention (see Fig. 1). Hence, the increase in LC α-tocopherol was inversely related to weight ($P < 0.01$), BMI ($P < 0.01$), SDS-BMI ($P < 0.01$), body fat ($P < 0.01$), FMI

Table 2
Characteristics of study population at baseline and during the weight loss treatment.

Characteristics	N	Baseline	2 months	6 months	P
Clinical parameters					
Weight (kg)	106	85.7 ± 1.66 ^A	81.5 ± 1.57 ^B	80.2 ± 1.58 ^C	<0.001
BMI (kg/m ²)	106	31.4 ± 0.49 ^A	29.7 ± 0.47 ^B	28.8 ± 0.49 ^C	<0.001
SDS-BMI	106	2.80 ± 0.05 ^A	2.55 ± 0.06 ^B	2.35 ± 0.07 ^C	<0.001
Waist circumference (cm)	97	99.0 ± 1.24 ^A	95.8 ± 1.16 ^B	90.8 ± 1.17 ^C	<0.001
Body fat (%)	93	35.8 ± 0.47 ^A	34.3 ± 0.50 ^B	33.2 ± 0.60 ^C	<0.001
Body fat (kg)	92	30.8 ± 0.80 ^A	28.1 ± 0.76 ^B	26.8 ± 0.82 ^C	<0.001
FMI (kg/m ²)	90	11.2 ± 0.28 ^A	10.2 ± 0.28 ^B	9.6 ± 0.30 ^C	<0.001
FFM (%)	93	64.2 ± 0.47 ^A	65.7 ± 0.50 ^B	66.8 ± 0.60 ^C	<0.001
Systolic blood pressure (mm Hg)	80	123.4 ± 1.60 ^A	118.7 ± 1.45 ^B	118.0 ± 1.45 ^B	0.002
Diastolic blood pressure (mm Hg)	80	72.6 ± 1.29 ^A	67.4 ± 1.23 ^B	67.1 ± 1.04 ^B	<0.001
Biochemical parameters					
Glucose (mmol/L)	97	4.63 ± 0.04 ^A	4.51 ± 0.05 ^B	4.54 ± 0.05 ^{A,B}	0.012
Insulin (µU/mL)	20	18.8 ± 3.00 ^A	14.5 ± 1.46 ^B	14.8 ± 1.58 ^B	0.047
Cholesterol (mmol/L)	106	4.01 ± 0.06 ^A	3.67 ± 0.07 ^B	3.77 ± 0.07 ^C	<0.001
HDL-cholesterol (mmol/L)	104	1.15 ± 0.03 ^A	1.05 ± 0.03 ^B	1.14 ± 0.03 ^C	<0.001
LDL-cholesterol (mmol/L)	104	2.34 ± 0.06 ^A	2.14 ± 0.06 ^B	2.17 ± 0.06 ^B	<0.001
Triacylglycerol (mmol/L)	106	1.01 ± 0.05 ^A	0.91 ± 0.05 ^A	0.86 ± 0.04 ^B	0.001
Apolipoprotein A1 (mg/dL)	73	117.14 ± 2.16 ^A	106.64 ± 1.86 ^B	112.79 ± 2.03 ^C	<0.001
Apolipoprotein B (mg/dL)	73	69.93 ± 2.06 ^A	65.32 ± 2.04 ^B	65.09 ± 2.32 ^B	<0.001
ApoB/apoA1 ratio	73	0.62 ± 0.19 ^{A,B}	0.63 ± 0.18 ^A	0.59 ± 0.19 ^B	0.002
C-reactive protein (mg/L)	58	3.26 ± 0.43 ^A	2.12 ± 0.23 ^B	2.12 ± 0.20 ^B	0.001
Physical condition					
Hand grip strength (kg)	107	29.12 ± 7.78 ^A	29.82 ± 8.10 ^A	30.51 ± 8.08 ^B	<0.001
Agility (seconds)	106	13.62 ± 1.53 ^A	13.03 ± 1.50 ^B	13.01 ± 1.28 ^B	<0.001
Cardiorespiratory endurance (periods)	98	3.07 ± 1.52 ^A	3.56 ± 1.82 ^B	3.77 ± 1.80 ^B	<0.001
Dietary intake					
Energy (kcal/d)	113	3336.87 ± 1613.99 ^A	–	2202.02 ± 625.56 ^B	<0.001
Carbohydrates (g/d)	113	362.9 ± 181.09 ^A	–	249.38 ± 74.33 ^B	<0.001
Proteins (g/d)	113	130.81 ± 59.27 ^A	–	108.55 ± 30.45 ^B	<0.001
Lipids (g/d)	113	151.04 ± 84.42 ^A	–	85.30 ± 34.83 ^B	<0.001
Total fibre (g/d)	113	27.05 ± 13.01 ^A	–	26.26 ± 9.91 ^A	0.553
Vitamin A (µg/d) ^a	90	486.26 ± 601.23 ^A	–	197.15 ± 314.49 ^B	<0.001
Vitamin E (mg/d) ^a	100	11.44 ± 10.81 ^A	–	6.03 ± 4.53 ^B	<0.001
β-Carotene (µg/d) ^a	108	3724.94 ± 4602.29 ^A	–	4997.34 ± 3882.13 ^B	0.014
Lycopene (µg/d) ^a	107	119.29 ± 168.66 ^A	–	152.86 ± 160.73 ^A	0.064

Results expressed as means ± SEM. Means in a row with different superscript letters are significantly different, $P < 0.05$ (general linear model with Bonferroni post hoc correction). BMI, body mass index; SDS-BMI, BMI standard deviation score; FMI, fat mass index; FFM, fat free mass; HDL-cholesterol, high-density lipoprotein cholesterol; LDL-cholesterol, low-density lipoprotein cholesterol; ApoB/apoA1 ratio, apolipoprotein B/apolipoprotein A1 ratio.

^a Determined from intake of dietary sources.

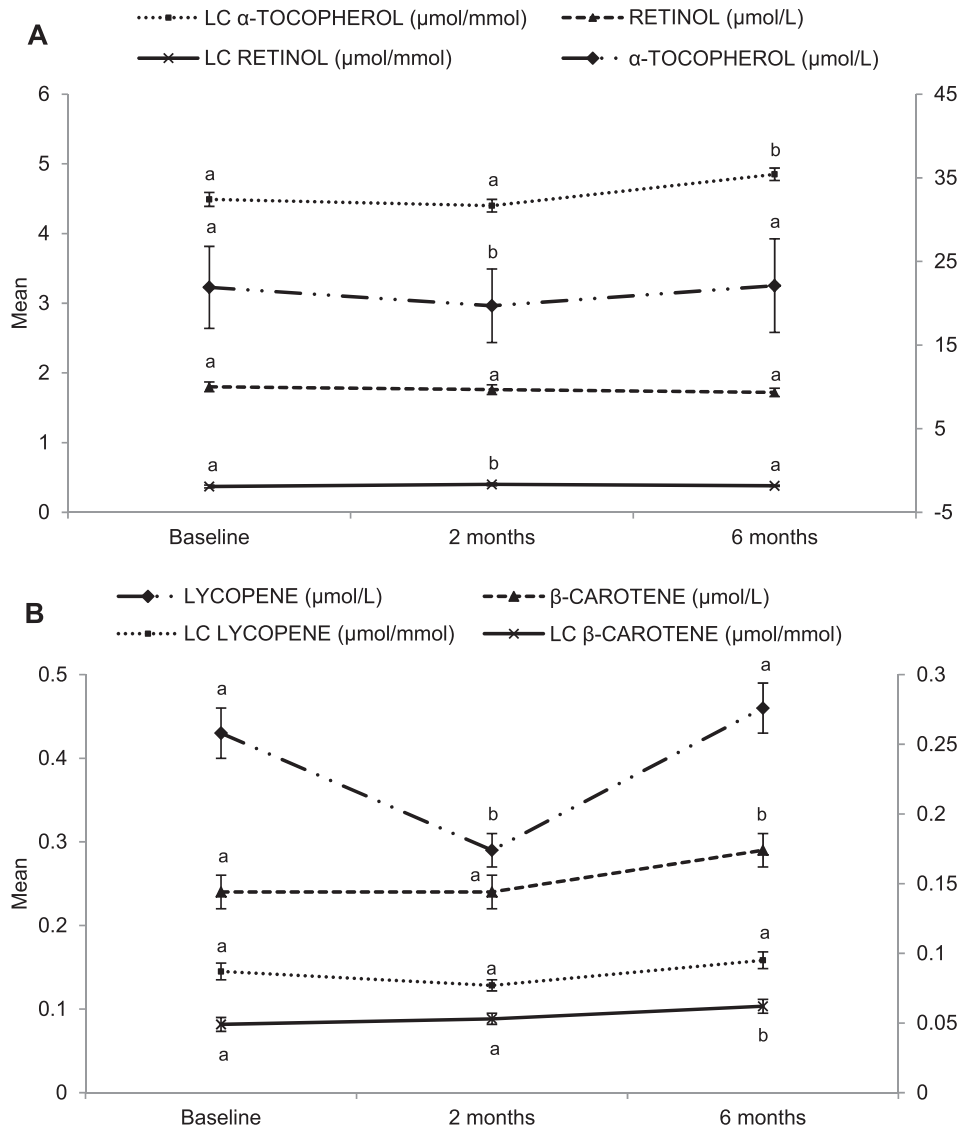


Fig. 1. Lipid-soluble antioxidant vitamin (A) and carotenoid (B) plasma levels. Values expressed as means \pm SD. The scale for LC α -tocopherol, retinol and LC retinol is 0–6; for α -tocopherol –5 to 45; for lycopene and β -carotene 0–0.5; and for LC lycopene and LC β -carotene 0–0.3. Means in a row with different superscript letters are significantly different, $P < 0.05$ (general linear model with Bonferroni post hoc correction). LC, lipid-corrected; α -tocopherol, $n = 103$; retinol, $n = 103$; lycopene, $n = 102$; β -carotene, $n = 101$; LC α -tocopherol, $n = 85$; LC retinol, $n = 85$; LC lycopene, $n = 84$; LC β -carotene, $n = 83$.

($P < 0.01$), cholesterol ($P < 0.001$), LDL cholesterol ($P < 0.05$) and TAG ($P < 0.001$). Conversely, the relationship observed between LC α -tocopherol and FFM was positive ($P < 0.01$). LC β -carotene concentration showed a significant and negative association with insulin ($P < 0.01$) and HDL cholesterol ($P < 0.001$).

In our study, the degree of weight loss in adolescents during the programme proposed was defined as the decrease in SDS-BMI. Changes in clinical and biochemical parameters, antioxidant vitamins and carotenoids, defined as a reduction in SDS-BMI at 6 months follow up, are given in Fig. 2. Adolescents were divided into three groups according to changes in SDS-BMI between baseline and 6 months of intervention. The *SDS-BMI decrease* > 0.5 group showed the largest decrease in anthropometric indicators of adiposity ($P < 0.001$) and biochemical parameters ($P < 0.05$), whereas the *SDS-BMI decrease* < 0.25 group presented the lowest reduction, or the opposite behaviour (Fig. 2A and B, respectively). Conversely, the highest increase in FFM was observed in the *SDS-BMI decrease* > 0.5 group and the lowest in the *SDS-BMI*

decrease < 0.25 group ($P < 0.001$). No differences were found between the weight loss groups in SBP, DBP, glucose, insulin, HDL cholesterol, CRP, apoA1 and the apoB/apoA1 ratio.

On the other hand, by comparing the baseline values with those obtained at 6 months of treatment in the same group of weight loss, it was found that the clinical and biochemical parameters, excepting insulin and HDL cholesterol, changed significantly in the *SDS-BMI decrease* > 0.5 group ($P < 0.05$). Should be noted that the indicators of general adiposity decreased and the FFM increased significantly even in the adolescents that had a lower weight loss (*SDS-BMI decrease* < 0.25) ($P < 0.05$).

In relation to antioxidant vitamin and carotenoid levels at 6 months (Fig. 2C), it was observed that the changes in LC retinol were independent to degree of weight loss, because there were no differences between groups or in the same group. When comparing the vitamin levels at 6 months with baseline values for each weight loss group, it was observed that LC β -carotene ($P = 0.034$) and LC α -tocopherol ($P = 0.001$) increased significantly in the adolescents

Table 3
Association between changes in plasma antioxidant vitamin and carotenoid levels and clinical and biochemical parameters at 2 and 6 months of treatment.

Parameters ^a	2 months			6 months		
	α -Tocopherol ^{a,b}	Retinol ^{a,b}	β -Carotene ^{a,b}	α -Tocopherol ^{a,b}	Retinol ^{a,b}	β -Carotene ^{a,b}
Weight (kg)	-0.162	-0.061	0.174	-0.347**	-0.146	-0.039
BMI (kg/m ²)	-0.193	-0.154	0.183	-0.380**	-0.174	-0.088
SDS-BMI	-0.153	-0.103	0.161	-0.398**	-0.141	-0.112
WC (cm)	0.243	0.107	0.084	-0.164	0.064	0.151
Body fat (%)	-0.252	0.216	-0.012	-0.444**	-0.162	-0.243
Body fat (kg)	-0.302	0.133	0.147	-0.412**	-0.147	-0.101
FMI (kg/m ²)	-0.284	0.066	0.128	-0.427**	-0.174	-0.151
FFM (%)	0.302	-0.216	0.012	0.444**	0.162	0.243
SBP (mm Hg)	-0.104	0.081	0.030	0.019	0.217	0.019
DBP (mm Hg)	0.272	-0.046	-0.192	0.215	0.084	0.254
Glucose (mmol/L)	0.132	-0.238	0.049	0.179	-0.194	0.056
Insulin (μ U/mL)	0.069	-0.025	-0.523	-0.199	0.219	-0.778**
Cholesterol (mmol/L)	-0.495**	-0.404*	0.156	-0.482***	-0.541**	-0.233
HDL-C (mmol/L)	-0.220	0.119	-0.288	-0.225	-0.188	-0.526***
LDL-C (mmol/L)	-0.418*	-0.266	0.244	-0.329*	-0.403*	-0.066
TAG (mmol/L)	-0.368*	-0.587***	-0.016	-0.505***	-0.520**	-0.084
ApoA1 (mg/dL)	-0.116	0.111	0.085	-0.121	-0.137	-0.226
ApoB (mg/dL)	-0.344	-0.269	0.281	-0.167	-0.455*	-0.140
ApoB/apoA1	-0.152	-0.213	0.176	0.122	-0.513*	-0.058
CRP (mg/L)	0.318	-0.235	-0.071	0.129	-0.003	0.133

Standardised regression coefficients adjusted for age, sex, SDS-BMI and Tanner stages at baseline, changes in intake of energy and corresponding vitamins or carotenoids at 6 months, and degree of physical activity; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ (linear regression model).

BMI (body mass index), $n = 49$; SDS-BMI (standard deviation score-BMI), $n = 49$; WC (waist circumference), $n = 49$; FMI (fat mass index), $n = 48$; FFM (fat free mass), $n = 48$; SBP (systolic blood pressure), $n = 44$; DBP (diastolic blood pressure), $n = 44$; HDL-C (high-density lipoprotein cholesterol), $n = 51$; LDL-C (low-density lipoprotein cholesterol), $n = 51$; TAG (triaclylglycerol), $n = 51$; ApoA1 (apolipoprotein A1), $n = 36$; ApoB (apolipoprotein B), $n = 36$; ApoB/apoA1 (apolipoprotein B/apolipoprotein A1 ratio), $n = 36$; CRP (C-reactive protein), $n = 36$. Weight, $n = 49$; body fat percentage, $n = 48$; body fat kg, $n = 48$; glucose, $n = 51$; cholesterol, $n = 51$; insulin, $n = 15$.

^a Changes in parameters between the baseline and 2 or 6 months of treatment.

^b Lipid-corrected values.

that experienced a decrease in SDS-BMI higher than 0.5, whereas LC lycopene improved in *SDS-BMI decrease 0.25–0.5* group ($P = 0.019$).

4. Discussion

Since plasma lipid-soluble vitamins and carotenoids are carried by lipoproteins [11,16] and given the relationship detected, in our study, between plasma lipids and changes in vitamin or carotenoid concentrations (data not shown), the effects of α -tocopherol, retinol, β -carotene and lycopene on biochemical and clinical parameters were analysed using their lipid-corrected levels [6].

Previous studies have reported that obese children present higher lipid concentrations [3,6] and peroxidation [5] than their normal weight counterparts. Antioxidant vitamins and carotenoids reduce lipid peroxidation and enhance LDL cholesterol resistance to oxidation [11,12]. In turn, there is evidence that whether adjusted and unadjusted for lipids, both β -carotene and α -tocopherol levels are lower in obese children [6]. The negative association confirmed, in this research, between changes in lipid concentration and changes in LC α -tocopherol, LC retinol and LC β -carotene suggests that the increase in plasma antioxidant vitamins and carotenoids may protect obese adolescents against oxidative stress.

In agreement with other studies, in which obese adults [23] and adolescents [24] were subjected to different dietary regimes, we found that HDL cholesterol decreased in the first stage of treatment. The subsequent recovery of this and apolipoprotein A1 (apoA1) was essential because they play an important role in removing the excess of cholesterol from tissues, consequently exerting an anti-atherogenic effect [2,22]. Current studies have suggested that the apoB/apoA1 ratio predicts CVD risk better than any traditional cholesterol index, such as total cholesterol or LDL cholesterol [25]. Since in this study, the apoB/apoA1 ratio decreased in the last stage of intervention, a reduction in the participants' risk of CVD could be reached.

Recently, insulin resistance and hyperinsulinemia have emerged as major concerns in overweight and obese children [3], since both may lead to impaired glucose tolerance and type-2 diabetes [26]. In addition to obesity itself, hyperinsulinemia and insulin resistance may play an important role as sources of oxidative stress [27] and they are related to reduced endogen and exogen antioxidants [6] in children and adolescents. In the present study, the decrease in plasma fasting insulin may be explained by the increase in β -carotene levels at 6 months of treatment, which is consistent with the results reported by other authors [6].

In relation to anthropometric indicators, we obtained interesting results for α -tocopherol, since the increase in LC α -tocopherol levels was associated to decrease in adiposity. These associations may be attributed to the strong antioxidant capacity of vitamin E [13,14]. The elevated protein oxidation and lipid peroxidation found in obese adolescents [5] may induce the production of inflammatory eicosanoids and cytokines [14], which suggests that improving α -tocopherol concentrations may represent an useful anti-inflammatory approach. In addition, the role of vitamin E as regulator of enzymes and gene activity [16] has recently been discovered. Hence, α -tocopherol may modulate genes with an important function in endocrine signalling, specifically in glucose and lipid metabolism [16].

Regarding the degree of weight loss, in previous studies, only obese children and adolescents with a decrease in SDS-BMI > 0.5 have been observed to present an improved CVD risk factor profile and intima-media thickness [28], an early marker of atherosclerosis. However, we found a decrease in plasma proatherogenic lipids also in the *SDS-BMI decrease 0.25–0.5* group. In addition, BMI, body fat and FMI decreased even in the group with lower weight loss. This suggests that adiposity indicators, especially those of general adiposity and pro-atherogenic lipids, are more influenced by degree of weight loss than blood pressure, glucose, CRP and apolipoproteins. These findings could be useful when setting

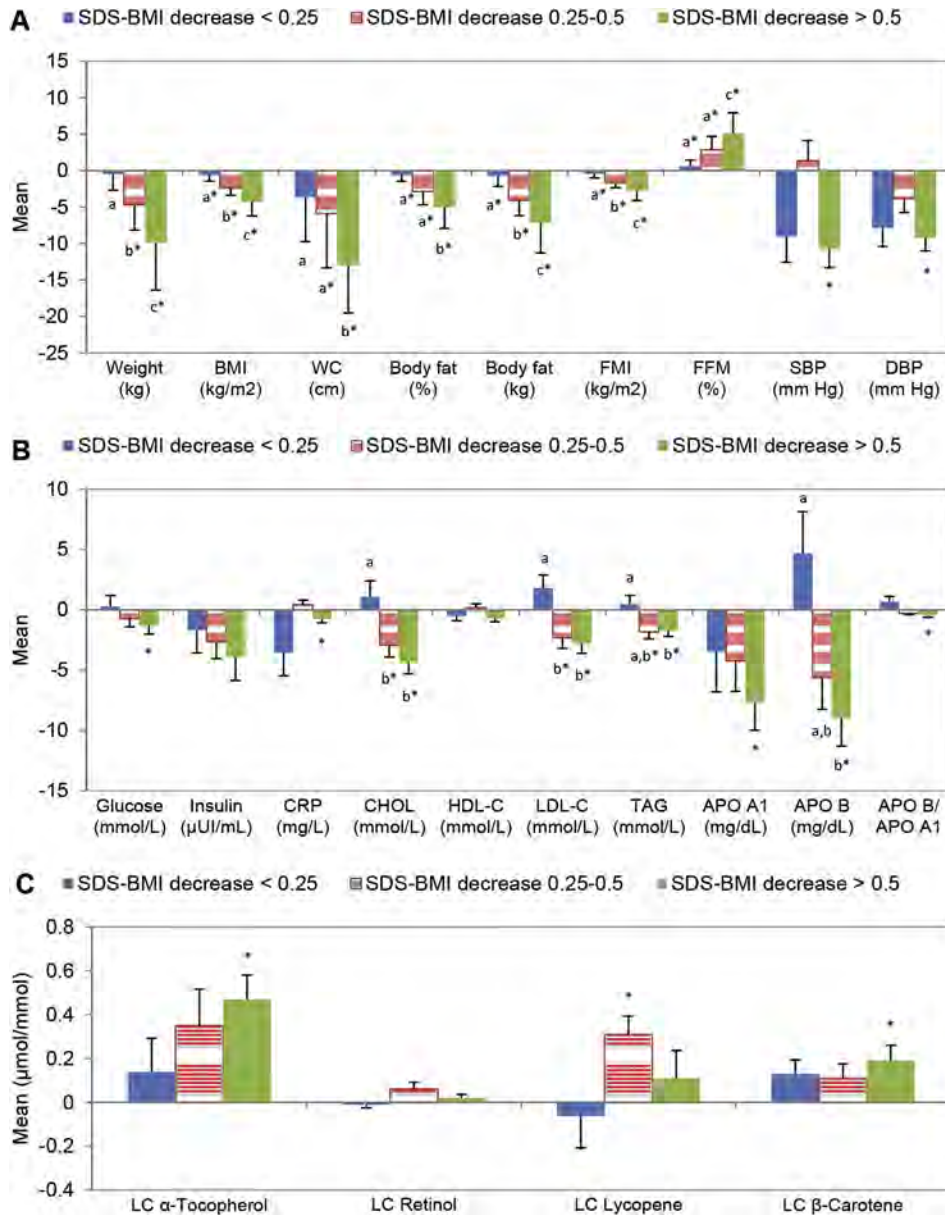


Fig. 2. Changes in clinical (A) and biochemical (B) parameters, lipid-soluble antioxidant vitamins and carotenoids (C) according to degree of weight loss. Changes in parameters between baseline and 6 months as defined by a reduction in BMI standard deviation score (SDS-BMI) at 6 months follow-up. Values expressed as means \pm SD. Models were adjusted for age, sex, SDS-BMI, Tanner stages and corresponding variables at baseline, changes in intake of energy and corresponding vitamins or carotenoids at 6 months, and degree of physical activity. Means in a row with different superscript letters are significantly different, *statistically significant differences in parameter changes at 6 months of treatment in relation to baseline, $P < 0.05$ (general linear model with Bonferroni post hoc correction). Means for glucose, Chol, HDL-C, LDL-C, TAG, APO B/APO A1, LC lycopene and LC β -carotene were multiplied by a factor of 10. BMI, body mass index; WC, waist circumference; FMI, fat mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; CRP, C-reactive protein; CHOL, cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; TAG, triacylglycerol; APO A1, apolipoprotein A1; APO B, apolipoprotein B; APO B/APO A1, apolipoprotein B/apolipoprotein A1 ratio; LC, lipid-corrected. Anthropometry, $n = 10/19/23$ (for SDS-BMI decrease < 0.25, SDS-BMI decrease 0.25–0.5 and SDS-BMI decrease > 0.5, respectively); blood pressure, $n = 15/23/29$; lipid profile and glucose, $n = 17/25/33$; insulin, $n = 4/7/5$; apolipoproteins and CRP, $n = 6/10/19$; LC α -tocopherol, $n = 10/16/22$; LC retinol, $n = 7/13/20$; LC β -carotene, $n = 8/17/23$ and LC lycopene, $n = 10/17/23$.

weight loss goals for obese adolescents with or without associated disorders.

In addition, it is interesting to note that weight losses greater than 0.5 of SDS-BMI were related to an increase in LC α -tocopherol and LC β -carotene levels. Furthermore, it was observed that the adolescents with intermediate decline of SDS-BMI (0.25–0.5) exhibit an increase in LC lycopene concentration at 6 months. These interactions were expected because it has been established that vitamin E and carotenoids may modulate several genes involved in glucose and lipid metabolism [15,16], fat storage [8] and inflammation in adipose tissue [9].

The reduction in adiposity, biochemical parameters and blood pressure observed in our study population are in line with other studies on children, showing that weight loss, as determined by a decrease in BMI or SDS-BMI, leads to an improvement in the CVD risk factor profile [29]. These results are of great importance since it has been demonstrated that atherosclerosis is initiated in childhood [30]; therefore, the changes observed in subjects' cardio-metabolic profile in our study might prevent or delay the onset of the CVD associated with obesity in adulthood.

The main limitation of this study was the absence of a control group. Consequently, it has not been possible to establish the

differences between obese and normal weight adolescents. However, as our initial aim was to analyse the evolution of the different indicators during the course of treatment and their relationship with weight loss, we consider the use of baseline values as control to be acceptable. The majority of the parameters studied showed a decrease during the intensive phase of treatment. After this phase, these parameters presented stable values or even experienced an unexpected increase. Consequently, it might be interesting to conduct a long-term study in order to evaluate the stability of the improvements achieved and optimise the duration and intensity of the programme. The periods established for medical controls should also be revised, since shorter periods between checks might favour correct observance of the instructions, especially when dealing with young population.

The present study has clearly demonstrated that lipid-corrected antioxidant vitamin and carotenoid levels changed significantly during the proposed treatment. The increase in lipid-corrected α -tocopherol, retinol and β -carotene plasma levels was associated with a reduction in adiposity and a clinically significant improvement in cardio-metabolic profiles.

The increase in lipid-soluble antioxidant vitamin and carotenoid plasma levels could be useful in childhood obesity treatment, since lipid-corrected α -tocopherol, β -carotene and lycopene plasma levels are related to greater weight loss. To our knowledge, no studies have been published to date which have analysed changes in lipid-soluble vitamins and carotenoids according to degree of weight loss. Consequently, we believe that these findings may be useful to establish unstudied relationships.

Further research is required to confirm the effects of lipid-soluble antioxidant vitamins and carotenoids on anthropometric indicators of adiposity, weight loss and cardio-metabolic profile in diverse populations.

Statement of authorship

M.G., R.M., G.L.B., M.M.M., A.I.C., E.M.B., A.M., J.A.M., L.M., J.M.G., J.W., J.C., A.M., M.C.L.S. and C.C. designed research; M.G. conducted research and wrote the paper; M.G. and J.M.P. analysed data; R.M., A.I.C. and M.C.L.S. did a critical review of the manuscript; C.C. and M.C.L.S. had primary responsibility for final content. All authors read and approved the final manuscript.

Conflict of interest

No conflicts of interest.

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