

## TITLE PAGE

**Title:** Total antioxidant capacity and oxidative stress after a 10-week dietary intervention program in obese children.

**Concise title:** Oxidative status after weight loss in obese children.

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## ABSTRACT

Dietary and serum total antioxidant capacity (TAC) are considered appropriate tools for investigating the potential healthy effects of dietary antioxidants consumed in mixed diets. The aim was to analyse the impact of a dietary intervention on macronutrient intakes and to evaluate the improvement on oxidative status after weight loss (WL) by

measuring dietary and serum TAC, and urinary F2-isoprostane levels as markers of oxidative stress. Forty-four overweight/obese children (mean age 11.5yr) were enrolled to undergo a 10-week WL program. They were dichotomized at the median of Body Mass Index-Standard Deviation Score (BMI-SDS) change, as high (HR) and low responders (LR) after intervention. Subjects were prescribed a fixed full-day meal diet, calculated according to their basal metabolic rate and physical activity levels. A validated food-frequency questionnaire was used to retrospectively calculate TAC and daily nutrient intake. The HR subjects were able to reduce anthropometric indices and to improve lipid and glucose profile. They also significantly diminished fat intake ( $p=0.013$ ).

Moreover, baseline serum TAC values did significantly predict the reduction in urinary F2 isoprostane [ $B= -0.236$  ( $-0.393$  to  $-0.078$ );  $p=0.014$ ] in the HR group after the WL program. Notably, changes in dietary TAC after the treatment were associated with a decrease in body weight after the 10-week intervention [ $B=-2.815$  ( $-5.313$  to  $-0.318$ ),  $p=0.029$ ] in the HR group. The  $-\Delta\text{SerumTAC}/\Delta\text{DietaryTAC}$  and the  $-\Delta\text{F2Isoprostane}/\Delta\text{DietaryTAC}$  ratios revealed that the relationships between oxidative markers and antioxidants dietary intake were more favourable in the HR than in the LR group.

*Conclusion:* Our study showed that a 10-week WL program was able to reduce adiposity indices in obese children. Moreover, after the intervention changes in dietary TAC and WL were significantly associated. Our result suggests that specific food with a high TAC content (such as fruits, vegetables and legumes) could be recommended to improve WL.

KEYWORDS: Obese children; Oxidative Stress; Antioxidant capacity; Obesity; Intervention; Isoprostane.

## INTRODUCTION

Oxidative stress, which is attributed to an excessive production of reactive oxygen species and/or an impaired antioxidant defence system in body cells, is often augmented in obese subjects [4,29,13]. Isoprostanes are products of free radical-catalyzed lipid peroxidation of arachidonic acid [11]. They are formed *in situ*, esterified to phospholipids, and released by phospholipases, into the plasma. They are subsequently removed from the plasma via the kidney, excreted in urine and used as reliable markers of oxidative stress. Thus, 8-epi-prostaglandin (PG)-F2 $\alpha$  or F2 isoprostane is reported to be greater in obese children [10,20]. Furthermore, F2 isoprostane is related to obesity complications such as atherosclerosis and insulin resistance in obese adults [8,16,48,52].

Furthermore, it has been suggested that dietary antioxidant intake is able to protect against oxidative damage and related inflammatory complications [9,39,40,5]. Moreover, given that the concentration of a single antioxidant may not reflect the total antioxidant value of food, the concept of dietary total antioxidant capacity (TAC) has emerged [47]. In fact, dietary TAC has been suggested as a tool for investigating the potential beneficial effects of dietary antioxidants occurring in mixed diets [3].

Since some dietary intervention programs in obese children demonstrated an oxidative status improvement after a weight loss (WL) process [27,14], we hypothesized that by modifying the dietary TAC, a 10-week moderate caloric restriction intervention could influence the oxidative status in obese children. Our aim was to analyse the impact of a WL intervention program on macronutrient intakes and oxidative status by measuring the dietary and serum TAC, as well as the urinary F2 isoprostane levels.

## **SUBJECTS AND METHODS**

### **Subjects**

For the study, 71 children between the ages of 7 and 15, categorized as overweight or obese according to the Cole *et al.* criteria [6], were invited to participate in an information session. Children were recruited from the Endocrinology Pediatric Units of the University of Navarra Clinic and Navarra's Hospital Complex in Pamplona, Navarra. They were Spanish, or foreign nationals being schooled in Spain for at least a year. Participants with a major psychiatric illness, significant neurological disease, bulimia nervosa, familiar hyperlipidemia or any sort of either major cardiovascular or respiratory complication, were excluded. Children and their parents signed a written

informed consent. The study protocol was performed in accordance with the Ethical standards of the Declaration of Helsinki, and was approved by the Ethics Committee of the University of Navarra (Reference Number 038/2009).

From the initial 71 volunteers, 54 successfully underwent baseline anthropometric measurements and 44 participants (22 boys, 22 girls) concluded the 10-week dietary intervention. The drop-out rate was 18.5% and the reasons for drop-out were: discouragement, social causes, forgetfulness, exams periods and inability to be accompanied by a family member to the dietician's weekly visit, as described in other children trials. Body Mass Index (BMI) was calculated from weight and height measurements. BMI z-scores or SDS (Standard Deviation Score) for sex and age were derived from Spanish reference data according to specific cut-off points for BMI [30]. The response of participants to the intervention program was based in changes in BMI-SDS. The sample (n=44) was dichotomized at the median of their BMI-SDS change (equal to 0.5) for analysing the response depending on the WL outcome. Thus, children who lost  $\geq 0.50$  BMI-SDS were considered "High Responders" (HR; n=22) and those who lost  $< 0.50$  BMI-SDS were considered "Low Responders" (LR; n=22) to the dietary intervention. Drop-outs were similar in both experimental groups.

#### Anthropometric, clinical and biochemical measurements

All anthropometric and biochemical measures were carried out at baseline and after the 10-week intervention, following standardized procedures [19]. Measurements were carried out by trained personnel, and all participants were barefoot and wearing light clothes. All measurements were performed three times and the final data were the mean from the values obtained.

Body weight was determined using a digital scale (TBF-410, TANITA, Tokyo, Japan). Height was measured using a stadiometer (Seca 220, Vogel & Halke, Germany). To measure height, hair ornaments were removed. The participants were asked to stand on the stadiometer with feet placed parallel and slightly apart and heels, buttocks, scapula and occipital head area touching the vertical board at the same time.

Waist circumference (WC) and hip circumference (HC) were measured using a non-stretchable measuring tape (Type SECA 200). The subjects were asked to stand erect on a flat surface in a relaxed position with both feet together. WC was measured as the smallest horizontal girth between the costal margins and the iliac crests at minimal

respiration. HC was taken as the greatest circumference at the level of the greater trochanter (the widest portion of the hip) on both sides. For both WC and HC, three measurements were made, and the mean of the three readings was taken as the final value. The waist-to-hip ratio and waist-to-height ratio were also calculated.

Blood pressure was measured using an electronic sphygmomanometer (Minimus II, Riester, Germany) on the right arm with a cuff that covered more than two-thirds of the upper arm, at a point midway between the olecranon and the acromion following WHO criteria. Blood pressure was measured twice and the blood pressure value was the mean of the two measurements.

Venous blood samples were obtained by trained nurses at the Hospital after an overnight fast. Glucose, insulin and lipid profile were determined by standard autoanalyzer techniques as described elsewhere [28]. Insulin resistance was calculated from fasting glucose and insulin values according to the homeostasis model assessment of insulin resistance (HOMA-IR) [24].

Serum TAC was determined, at baseline and at the end of the treatment, based on the Trolox equivalent antioxidant capacity assay using a colorimetric commercial kit (Cayman Chemical Corporation, Ann Arbor, MI, USA). To determine the level of urinary F2 isoprostane the first void of the morning urine was collected in plastic vessels for 3 consecutive days. Morning urine -rather than 24-hour urine collection- was obtained based on previous data which indicated that 24-hour measurements expressed per mg creatinine were not statistically different from values obtained from morning urine [50]. Urinary F2 isoprostane was measured using an ELISA kit according to the manufacturer's protocol (Oxford Biomedical Research, Oxford, MI, USA), and was expressed in ng/mg creatinine.

In addition to the anthropometry and biochemical determinations, children's body composition was measured before and after the intervention program using bioelectrical impedance analysis-BIA equipment (TBF-410, TANITA, Tokyo, Japan). Pubertal development was assessed according to the five established Tanner stages [49]. Each stage describes breast and pubic hair development in girls and genital and pubic hair development in boys.

Information on levels of physical activity was collected with the “Seguimiento Universidad de Navarra” (SUN) validated questionnaire and total metabolic equivalents-minutes per day (METs-min/day) for each participant were derived from the different physical activities indicated and their typical average energy expenditure, as described elsewhere [45].

### **Dietary treatment and intake assessment**

Dietary follow-ups, weight-control and nutritional education were performed weekly through individual sessions with a Registered Dietician (RD). The adherence to the ten-weekly appointments with the RD was 93% in all participants. Subjects were prescribed a fixed full-day meal diet, calculated according to their basal metabolic rate and physical activity levels [45]. Energy expenditure was estimated taking into account basal metabolism using the Schofield equation [46,31], according to sex and body growth period. Children and their parents received personal training in nutritional and physical education throughout the whole intervention period. In all cases, diets were not lower than 1500 kcal per day and not higher than 2000 kcal/d. Dietary intake information was obtained at baseline and at the end of the intervention, with the semiquantitative 136-item “Seguimiento Universidad de Navarra” (SUN) food-frequency questionnaire (FFQ), previously validated and applied in children [23,22,32]. Daily food consumption was estimated as frequency x portion size for each consumed food item. Nutrient intake was estimated using an *ad hoc* computer program specifically developed for this aim, which displays the latest available information, included in the food composition tables for Spain.

Furthermore, dietary TAC value was calculated based on the SUN FFQ by adding TAC values from the ferric reducing-antioxidant power assay (FRAP) of each food, as previously reported [17,18,34,35,44,37] and was expressed as TAC in mmol/100g of food. To assign a value to TAC-providing foods not available in previous reports, the data for a similar food item (e.g., same botanical group) were used as a proxy. When TAC values of cooked food were not available, TAC values of fresh food were used to calculate dietary TAC. The mean of TAC values of food contained in each item of the SUN FFQ was used to calculate the dietary TAC value from this questionnaire [38].

### **Statistical analyses**

Results are shown as mean±standard error of the mean (SEM). Unpaired and paired *t*-tests were used as appropriate to assess anthropometric and other studied variables at baseline and after the WL intervention. Multivariable lineal regression analyses were performed to estimate associations between basal serum TAC and the change in urinary F2 isoprostane, and between dietary TAC changes and WL, after adjustment for potential confounders when indicated. Confidence intervals (95% CI) are used to describe lineal regression coefficient (B) values. The following potential confounders were considered: sex, Tanner stage, baseline BMI-SDS, total energy intake or physical activity levels as indicated. Partial correlations were performed after the 10-week dietary intervention to further explain relationships between dietary TAC and consumption of several food items. Statistical analyses were performed using SPSS for Windows 15.0 software (SPSS Inc., Chicago, IL). A *p*-value less than 0.05 is considered as statistically significant.

## **RESULTS**

44 obese children (mean age 11.5 yr), with a BMI-SDS equal to 4.0 and 37.3% of fat mass were enrolled in the program. They were distributed in HR and LR groups according to the response (change in BMI-SDS) (Table 1). The HR and LR subjects presented similar anthropometric parameters and plasma profile at baseline. Serum and dietary TAC values and urinary F2 isoprostane as markers of oxidative stress from participants are also indicated in Table 1. With regard to physical activity no differences between both groups were observed after the intervention program (HR 0.97±5.90 METs-min/day and LR -1.01±4.89 METs-min/day; *p*=0.871 and *p*=0.840 respectively, data not shown).

Our obese children (*n*=44) did exceed most of the dietary reference intakes at baseline, except for fibre. Moreover, figure 1 shows that there are no differences in macronutrient intake (expressed as %E) between groups. After the intervention, there was a tendency to lower energy and macronutrients intake in both groups (Table 2). Interestingly, total fat intake was significantly lower (*p*=0.013) after the 10-week WL program in the HR group. No association was found between fat intake and WL or oxidative status markers in our trial. However, a strong association between dietary TAC and consumption of vegetables, cereals, fruits, vitamin C and folic acid was found in the HR group, regardless of BMI-SDS and total energy intake (Table 4).

Consequently, significant changes were observed in anthropometric and clinical parameters in the HR group (Table 3). The mean WL in the HR group was  $-3.73 \pm 0.24$  kg ( $p < 0.001$ ). Moreover, a significant reduction in BMI-SDS ( $-0.78 \pm 0.07$ ;  $p < 0.001$ ), body fat mass ( $-2.95 \pm 0.65\%$ ,  $p < 0.001$ ), and waist circumference ( $-5.40 \pm 0.55$  cm,  $p < 0.001$ ) were seen in HR subjects. The decrease in adiposity measurements in the HR group was associated with a reduction in serum glucose ( $-6.00 \pm 1.27$  mg/dL,  $p < 0.001$ ), total cholesterol ( $-20.95 \pm 5.02$  mg/dL,  $p < 0.001$ ), insulin levels ( $-4.53 \pm 1.59$   $\mu$ U/mL,  $p = 0.010$ ) and HOMA-IR ( $-1.23 \pm 0.37$ ,  $p = 0.003$ ). But, no statistical associations between oxidative status markers and changes in adiposity indices or biochemical parameters were found.

In a multivariable linear regression, basal serum TAC values did significantly predict the change in urinary F2 isoprostane after the WL [ $B = -0.236$  ( $-0.393$  to  $-0.078$ );  $p = 0.014$ ] in the HR group, after adjusting for potential confounders. Moreover, changes in dietary TAC after the treatment were associated with a decrease in body weight after the 10-week intervention [ $B = -2.815$  ( $-5.313$  to  $-0.318$ ),  $p = 0.029$ ] in the HR group.

Interestingly, the reduction in the dietary TAC in the HR group was slightly greater than in the LR group, but the decrease in serum TAC was lower (Table 3). The  $-\Delta$ serum TAC/ $\Delta$ dietary TAC ratio was similar in HR and LR subjects (Figure 2), but, the  $-\Delta$ F2 isoprostane/ $\Delta$ dietary TAC ratio was quite different ( $p = 0.026$ ) between HR and LR groups, being more favourable in HR subjects.

## **DISCUSSION**

Our study was designed to evaluate oxidative status changes in obese children after a WL intervention program by assessing dietary and serum TAC, and F2 isoprostane levels.

The WL program consisted in a moderate calorie restriction, not to interfere with children growth, which was able to reduce anthropometric indices and to improve lipid and glucose profile as reported elsewhere [43]. Previous studies have shown that an improvement in body composition and cardiometabolic risk can be seen with a BMI-SDS reduction of  $\geq 0.25$  in obese children, while greater benefits occurs when losing at least 0.5 BMI-SDS [12]. Our results suggest that a WL intervention as short as 10-week, can improve metabolic syndrome parameters in obese children, as seen in other studies [7,42].



Initially, a higher macronutrient intake especially for fat intake was found in our population compared to dietary reference intakes. Similar findings are reported for obese children in other trials [15,21]. After the WL intervention program, a modest reduction in fat intake was observed in the HR group, as observed in several intervention studies [53,54].

There is evidence that the dietary intake information derived from a FFQ is appropriate to calculate the dietary TAC in a reliable manner in children and adults [33,41]. Dietary TAC has been highly related to the consumption of some specific food groups as vegetables, fruits and legumes [41], which are an important source of dietary antioxidant nutrients or components [1,17].

Furthermore, a recent study showed that dietary TAC is positively associated with plasma TAC in healthy young adults [38], suggesting that dietary TAC may constitute a useful tool in antioxidant intake assessment, although this outcome was not found in our trial. In our obese children it seems that baseline serum TAC predicts the reduction in urinary F2 isoprostane after the WL program.

No changes in oxidative stress markers were found in our obese population after the WL program. We could not find studies performed in children. In adults, Tsai *et al.* (2009) showed similar findings, a 4% reduction in body weight did not change either plasma or urinary F(2)-isoprostane [51]. Moreover, Melissas *et al.* (2006) did not observe any changes in serum TAC after surgical weight reduction [25].

Nevertheless, after the intervention changes in dietary TAC and WL were significantly associated in the HR group. This could suggest that specific food with a high TAC content (such as fruits, vegetables and legumes) are to be recommended to improve WL. In a similar way, Bahadoran *et al.* (2012), showed that a higher intake of dietary antioxidants also resulted in lower body weight and abdominal fat gain in adults [2]. Moreover, Puchau *et al.* (2010) showed an inverse association between adiposity (BMI-SDS, total body fat) and dietary TAC in obese children [36]. It seems that the potential effects of food antioxidants may occur through modification of lipids and carbohydrates metabolism, increased insulin sensitivity, and regulation of both appetite and adipocytokines [26].

Strengths of our study include: 1) measurements in young subjects less exposed to chronic oxidative stress; 2) the overweight/obese subjects in the HR group, achieved substantial WL (higher than 5% of initial body weight) after a short dietary intervention; 3) a standardized intervention with similar dietary conditions applied to a relatively homogeneous group; and 4) the assessment of markers of oxidative stress at baseline and after the intervention. On the other hand, some weaknesses of the study are: 1) our design a “case-only” approach without normal weight subjects; 2) the limited number of children per group; 3) bias in reporting of food intake; and 3) the pre-pubertal stage of the volunteers, with an intense growth and endocrine changes which may influence the outcome.

In summary, our study demonstrates that a 10-week WL program had a positive impact on changes in body composition and biochemical profile in obese children (HR group). Our data suggest that dietary TAC, as a measure of antioxidant intake, could be a potential marker of diet quality in obese children with a successful response to a WL program.

DISCLOSURE STATEMENT: the authors have nothing to disclose.

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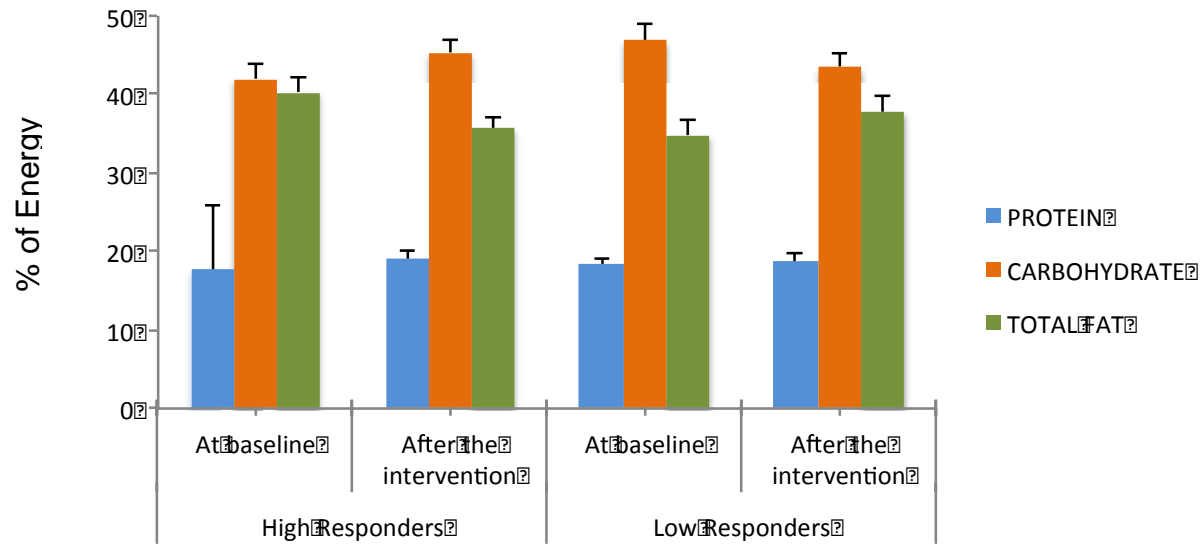


Figure 1: Mean daily macronutrient intake, expressed as percentage of energy, at baseline and after the treatment according to the intervention response (HR, n=22; LR, n=22).

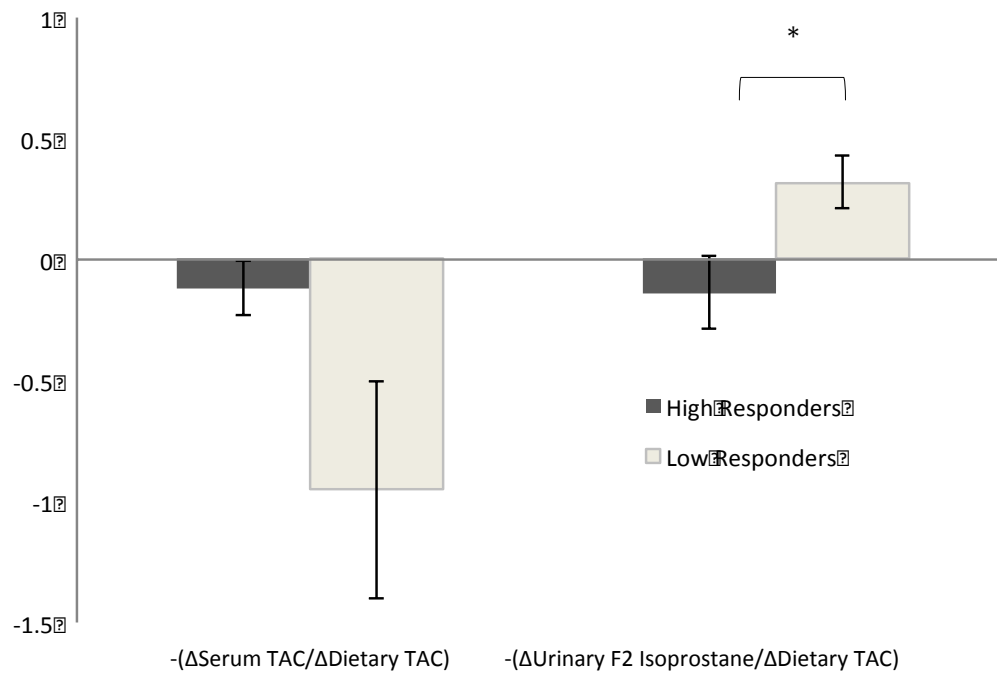


Figure 2: Ratio between changes in serum TAC or urinary F2 Isoprostane and dietary TAC after the intervention. \*p-value <0.05

Table 1: Baseline characteristics of overweight/obese children, according to the intervention response.

	All participants n=44	High Responders n=22	Low Responders n=22	p-value
Age (y)	11.52±0.39	11.23±0.59	11.82±0.52	0.458
Sex (boys/girls)	22/22	14/8	8/14	0.073
Tanner stage (I/II/III)	11/28/5	7/12/3	4/16/2	0.618
Weight (Kg)	72.64±3.09	68.16±3.66	77.11±4.87	0.149
Height (m)	1.53±0.02	1.51±0.03	1.56±0.03	0.252
BMI-SDS	4.00±0.31	4.01±0.39	3.99±0.49	0.975
Body Fat (%)	37.30±1.20	35.60±1.77	39.00±1.58	0.157
Waist circumference (cm)	91.12±1.43	89.89±2.11	92.34±1.96	0.400
Hip circumference (cm)	101.54±1.88	98.50±2.23	104.58±2.93	0.106
Waist to hip ratio	0.90±0.01	0.91±0.01	0.89±0.01	0.175
Waist to height ratio	0.59±0.01	0.59±0.01	0.59±0.01	0.967
Diastolic BP (mmHg)	73.46±2.55	75.40±3.25	71.62±3.94	0.466
Systolic BP (mmHg)	129.54±2.73	127.95±4.39	131.05±3.40	0.578
Heart rate (bpm)	82.44±1.90	83.63±2.85	81.30±2.57	0.547
Serum glucose (mg/dL)	92.25±1.01	93.00±1.74	91.50±1.04	0.464
Serum insulin (μU/mL)	17.94±1.33	17.92±1.64	17.95±2.13	0.992
HOMA-IR	4.10±0.31	4.13±0.38	4.07±0.49	0.929

Serum total cholesterol (mg/dL)	174.61±4.91	179.77±7.84	169.45±5.89	0.299
Serum triglycerides (mg/dL)	98.98±8.13	88.05±7.85	109.91±14.06	0.184
Serum HDL cholesterol (mg/dL)	49.73±1.87	51.27±2.86	48.18±2.42	0.415
Serum LDL cholesterol (mg/dL)	101.82±3.82	105.59±6.09	98.05±4.61	0.329
Serum TAC (mM/Trolox)	1.52±0.16	1.46±0.23	1.66±0.20	0.516
Dietary TAC (mmol)	5.13±0.73	6.15±1.46	5.31±0.67	0.602
Urinary F2 Isoprostane (ng/mgCr)	3.45±0.33	3.39±0.25	3.27±0.31	0.767

Variables are expressed as mean ± SEM. BMI-SDS: Body Mass Index-Standard Deviation Score; BP: Blood Pressure; HOMA-IR: Homeostasis Model Assessment of Insulin Resistance; TAC: Total Antioxidant Capacity.

- 1: p-value for the differences after the intervention in the HR group compared to the values of this group at baseline.
- 2: p-value for the differences after the intervention in the LR group compared to the values of this group at baseline.
- 3: p-value for the differences after the intervention between the HR and the LR group.

Table 2: Total energy and macronutrient intake at baseline (n=44) and after the treatment according to the intervention response (HR, n=22; LR, n=22).

	At baseline	After intervention		p-value <sup>1</sup>	p-value <sup>2</sup>	p-value <sup>3</sup>	p-value <sup>4</sup>
	All participants n=44	High Responders n=22	Low Responders n=22				
Total Energy Intake (kcal/d)	2667±147	2333±164	2228±172	0.661	0.056	0.422	0.384
Carbohydrates (g/d)	297±20	268±24	242±22	0.426	0.337	0.244	0.871
Proteins (g/d)	117±6	109±10	101±7	0.478	0.348	0.333	0.699
Total Fats (g/d)	112±8	92±7	95±10	0.750	0.013	0.972	0.068
Saturated fatty acids (SFA) (g/d)	35±2	28±3	29±3	0.801	0.008	0.736	0.087
Monounsaturated fatty acids (MUFA) (g/d)	46±3	40±3	42±5	0.644	0.030	0.764	0.107
Polyunsaturated fatty acids (PUFA) (g/d)	16±2	12±1	13±2	0.723	0.111	0.871	0.128
Cholesterol (mg/d)	470±27	437±43	394±31	0.421	0.266	0.461	0.714
Fiber (g/d)	24±2	25±2	25±2	0.874	0.918	0.428	0.665

Variables are expressed as mean ± SEM.

1: p-value for the intake after the intervention, comparing between the HR and the LR group.

2: p-value for the differences after the intervention in the HR group.

3: p-value for the differences after the intervention in the LR group.

4: p-value for the differences after the intervention between the HR and the LR group.



Table 3: Changes in the HR and the LR group after the 10-week intervention program.

	High Responders n=22	Low Responders n=22	p-value <sup>1</sup>	p-value <sup>2</sup>	p-value <sup>3</sup>
ΔWeight (Kg)	-3.73±0.24	-0.65±0.35	<0.001	0.074	<0.001
ΔHeight (m)	0.01±0.00	0.01±0.00	<0.001	0.001	0.327
ΔBMI-SDS	-0.78±0.07	-0.20±0.49	<0.001	<0.001	<0.001
ΔBody Fat (%)	-2.95±0.65	-0.79±0.32	<0.001	0.024	0.006
ΔWaist circumference (cm)	-5.40±0.55	-2.28±0.75	<0.001	0.006	0.002
ΔHip circumference (cm)	-3.27±0.38	-0.85±0.65	<0.001	0.205	0.003
ΔWaist to hip ratio	-0.03±0.01	-0.01±0.01	<0.001	0.054	0.230
ΔWaist to height ratio	-0.04±0.00	-0.02±0.00	<0.001	0.002	0.001
ΔDiastolic BP (mmHg)	-5.3±4.5	-3.9±3.6	0.259	0.281	0.816
ΔSystolic BP (mmHg)	-2.1±5.4	-5.4±4.1	0.695	0.211	0.636
ΔHeart rate (bpm)	-7.6±3.2	-1.8±3.0	0.028	0.549	0.199
ΔSerum glucose (mg/dL)	-6.00±1.27	-5.82±1.74	<0.001	0.003	0.933
ΔSerum insulin (μU/mL)	-4.53±1.59	0.62±1.35	0.010	0.649	0.018
ΔHOMA-IR	-1.23±0.37	-0.07±0.32	0.003	0.822	0.022
ΔSerum total cholesterol (mg/dL)	-20.95±5.02	-0.05±3.87	<0.001	0.898	0.002
ΔSerum triglycerides (mg/dL)	-16.82±8.08	0.14±12.53	0.050	0.991	0.262
ΔSerum HDL cholesterol (mg/dL)	-4.76±1.28	-3.09±0.99	0.001	0.005	0.305
ΔSerum LDL cholesterol (mg/dL)	-13.24±3.93	0.91±3.50	0.003	0.798	0.010
ΔDietary TAC (mmol)	-1.30±1.44	-0.36±0.76	0.377	0.639	0.570

$\Delta$ Serum TAC (mM/trolox)	-0.13 $\pm$ 0.20	-0.53 $\pm$ 0.29	0.523	0.095	0.287
$\Delta$ Urinary F2 Isoprostane (ng/mgCr)	-0.22 $\pm$ 0.28	0.15 $\pm$ 0.26	0.448	0.575	0.553

Variables are expressed as mean  $\pm$  SEM. BMI-SDS: Body Mass Index-Standard Deviation Score; BP: Blood Pressure; HOMA-IR: Homeostasis Model Assessment of Insulin Resistance; TAC: Total Antioxidant Capacity.

- 1: p-value for the differences after the intervention in the HR group compared to the values of this group at baseline.
- 2: p-value for the differences after the intervention in the LR group compared to the values of this group at baseline.
- 3: p-value for the differences after the intervention between the HR and the LR group.

Table 4: Partial correlations between dietary TAC (mmol), estimated from the FFQ, and the intake of antioxidant nutrients or food groups after the 10-week dietary intervention, adjusted by BMI-SDS and daily energy intake.

	After 10-week intervention			
	High Responders n=22		Low Responders n=22	
	r	p value	r	p value
Vegetables (g/d)	0.605	0.017	0.295	0.285
Cereals (g/d)	0.586	0.022	0.029	0.919
Fruits (g/d)	0.567	0.028	0.559	0.030
Vitamin C (mg/d)	0.751	0.001	0.592	0.020
Folic Acid (mg/d)	0.726	0.002	0.707	0.003