TITLE: Decreased cardiotrophin-1 levels are associated with a lower risk of developing the metabolic syndrome in overweight/obese children after a weight loss program.

AUTHORS NAMES AND AFFILIATIONS:

Tara Rendo-Urteaga[1*], Sonia García-Calzón[1*], Eduardo Martínez-Ansó[2], María Chueca[3], Mirentxu Oyarzabal[3], María Cristina Azcona-Sanjulián[4], Matilde Bustos[2], María Jesús Moreno-Aliaga[1,5], J. Alfredo Martínez[1,5], Amelia Marti[1,5].

1. Department of Nutrition, Food Science and Physiology, University of Navarra, Pamplona, Spain.
2. Division of Hepatology and Gene Therapy, Centre for Applied Medical Research (CIMA), Clínica Universidad de Navarra and Medical School, University of Navarra, Pamplona, Spain.
3. Pediatric Endocrinology Unit, Complejo Hospitalario de Navarra, Pamplona, Spain.
4. Pediatric Endocrinology Unit, Department of Pediatrics, Clínica Universidad de Navarra, Pamplona, Spain.
5. CIBER Fisiopatología de la Obesidad y Nutrición (CIBERobn), Instituto de Salud Carlos III, Madrid, Spain.

* These two authors have equally contributed to the present work.

CORRESPONDING AUTHOR: Dr. Amelia Marti del Moral (amarti@unav.es). Department of Nutrition, Food Science and Physiology. University of Navarra. C/Irunlarrea s/n CP31008 Pamplona, Navarra, Spain. Telephone: 0034 948 425600; Fax: 0034 948 425619

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ABSTRACT

Objective: Cardiotrophin-1 (CT-1) shares some similarities with other cytokines, and participates in the control of energy metabolism. Higher circulating levels are observed in obese humans, but little information is gathered in weight loss (WL) programs. Therefore, we aimed to investigate the association of serum CT-1 levels with metabolic variables and the risk of developing metabolic syndrome (MetS) after a WL program in overweight/obese children.

Subjects and Methods: Forty-four overweight/obese children (mean age 11.5 yr; 50% males) undergoing a 10-week WL program were enrolled. Subjects were dichotomized at the median of Body Mass Index-Standard Deviation Score (BMI-SDS) change, as high and low responders after intervention.

Results: CT-1 levels were significantly reduced (-48 fmol/mL, p=0.043) in the high responder group after the WL program. They had significantly lower body weight (-3.7 kg, p<0.001), body fat mass (-8%, p<0.001), BMI-SDS (-0.78, p<0.001) and waist circumference (-5.4 cm, p<0.001), and a significant improvement in lipid and glucose profiles (p<0.05). Interestingly, decreased CT-1 levels significantly predicted changes in total cholesterol (41%) and LDL-cholesterol (28%). Moreover, in our participants the lower the CT-1 levels, the higher the reduction in MetS risk components, after the 10-week intervention, (*p*-ANCOVA=0.040, *p*-trend=0.024).

Conclusion: We showed, for the first time, a reduction in serum CT-1 levels after a WL program and this decrease in CT-1 was strongly associated with a reduction in cholesterol levels and in MetS risk factors in overweight/obese children. Our findings may suggest that CT-1 could be an indirect marker for the diagnosis of MetS in this population.

KEY WORDS: Child; Intervention studies; Obesity; Cytokine.

ABBREVIATIONS: Weight loss, WL; Body Mass Index, BMI; Body Mass Index-Standard Deviation Score, BMI-SDS; Cardiotrophin-1, CT-1; Interleukin-6, IL-6; Ciliary Neurotrophic Factor, CNTF; Metabolic Syndrome, MetS; High Responder, HR; Low Responder, LR; Lipid Accumulation Product, LAP; Waist Circumference, WC.

1. INTRODUCTION

The prevalence of childhood obesity has almost tripled in US children and adolescents since 1980 [1] and in some countries there is an age-specific stabilization in these elevated values [2,3]. In Spain, a national survey in 2007 showed that 10.3% of the population aged 2-15 yr was obese and 18.8% was overweight [4]. The factors associated with overweight and obesity are complex, such as eating habits, daily physical activity and social, environmental and biological determinants. In fact, nutritional interventions are a valuable strategy to face this serious problem [3,5].

A successful weight loss (WL) program in overweight and obese children and adolescents is usually accompanied by a general metabolic improvement and a reduction in several cardiovascular risk factors [6-9]. Specifically, in intervention studies with 9-13 yr obese children, increased circulating adiponectin levels and lower HOMA-IR [8,10-12] were described, together with a reduction in fat mass, Body Mass Index (BMI) or Body Mass Index-Standard Deviation Score (BMI-SDS). Previous studies have shown that an improvement in body composition and cardiometabolic risk can be seen with a BMI-SDS reduction of ≥0.25 in obese adolescents, while greater benefits accrue from losing at least 0.5 BMI-SDS [6].

Concerning changes in blood lipids, a recent systematic review with meta-analysis on the effectiveness of lifestyle interventions in obese children, showed that lifestyle intervention did significantly lower total cholesterol compared with no treatment (-0.40 mmol/L) in 440 obese children aged 8-16 yr [5]. A similar effect was found for triglycerides, the pooled mean difference being -0.20 mmol/L [5].

In recent years, cardiotrophin-1 (CT-1) has emerged as a new player in the control of energy metabolism potentially linked to obesity and type 2 diabetes [13]. CT-1 is a 201 amino acid protein member of the interleukin-6 (IL-6) superfamily of cytokines. It

mediates a pleiotropic set of survival effects through a receptor system; consisting of glycoprotein 90 or leukemia inhibitory factor receptor beta (LIFRb) and a common signal transducer, the glycoprotein 130 (gp130) [14]. CT-1 is expressed in a variety of organs from human heart to liver, being nutritionally regulated [13]. It plays a role in the control of energy metabolism sharing some similarities with leptin and other cytokines such as IL-6 or CNTF (ciliary neurotrophic factor) [13,15]. Interestingly, in animals CT-1 activates fat utilization, displays glucose-lowering activity and also shows anorexigenic properties [13]. Thus, experimental data are promising for the therapeutic implication of CT-1 in obesity [16].

Endogenous CT-1 has prolonged stability in whole blood, hence permitting its development in the routine clinical investigation of patients [17]. It seems that circulating CT-1 could be considered as a promising biomarker in patients with cardiovascular diseases and the metabolic syndrome (MetS). However, few studies have been performed in obese human subjects. Two studies showed that obese adults had higher circulating CT-1 levels than normal-weight subjects [18,19] and in overweight adolescents no differences in plasma CT-1 were found when compared to control subjects [20]. There is also no information about the effect of a WL program in CT-1 levels. Thus, our aim was to evaluate changes in serum CT-1 levels after a WL program in overweight/obese children and to investigate the relationship with the risk of developing the MetS and other metabolic parameters. We hypothesized that decreased serum CT-1 levels may predict improvement in metabolic outcomes after a WL program in overweight/obese children, since it has been suggested that the high levels of CT-1 in obese subjects could be a protective mechanism to counteract the emergence of obesity-related alterations [13].

2. SUBJECTS AND METHODS

2.1. Subjects

In the study, 71 children between 7 and 15 yr and classified as overweight or obese according to the Cole *et al.* criteria [21] were invited to participate in the information session. Children were recruited from the Endocrinology Pediatric Units of the University of Navarra Clinic and Navarra's Hospital Complex in Pamplona, Navarra. All of them were Spanish or schooling foreigners for at least one year in Spain. Participants with a major psychiatric illness, significant neurological disease, bulimia nervosa, familial 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.

Forty-four participants (22 boys, 22 girls) concluded the 10-week dietary intervention (drop-out rate 18.5%) during two different periods (from April to June and from September to December, 2010) and were assessed for WL after the moderate calorie restriction treatment. BMI-SDS was calculated as a function of the subject's obesity degree when compared with BMI local reference standards [22], which is adjusted for sex and age and it is optimal for assessing adiposity in children. The response of participants to the WL program was based on changes in BMI-SDS. Subjects were dichotomized at the median of BMI-SDS (equal to 0.5). Those who lost >0.50 BMI-SDS were considered as "High Responders" (HR; n=22) and those who lost ≤0.50 BMI-SDS as "Low Responders" (LR; n=22) to the program. Previous studies have shown that an improvement in body composition and cardiometabolic risk can be seen with a BMI-SDS reduction of ≥0.25 in obese adolescents, while greater benefits accrue from

losing at least 0.5 BMI-SDS [6]. Baseline clinical and anthropometric characteristics of HR and LR are shown in table 1.

2.2. Dietary treatment

The child accompanied by his/her parent or tutor, received weekly individual sessions with a registered dietician for dietary follow-up, weight-control and nutritional education. The adherence to the ten weekly appointments with the registered dietician was 93% in the total sample. The study protocol consisted of a moderate calorie-restricted diet, calculated according to children's obesity degree [23], which did not interfere with the children's body growth.

Therefore, subjects were prescribed a diet based on a fixed full-day meal plan, calculated according to basal metabolic rate. Energy Expenditure was estimated taking into account basal metabolism using the Schofield equation, according to sex [24] and body growth period [25]. In all cases, diets were not lower than 1300 kcal and not higher than 2200 kcal. Children and their parents received personal training in nutritional and physical education throughout the intervention period. Identical personal physical training was also achieved.

2.3. Anthropometric, clinical and biochemical measurements

All anthropometric and biochemical measures were carried out at baseline and after the 10-week intervention, following validated procedures. Measurements were performed by trained personnel, in a wide space and all participants were barefoot and wore light clothing. All measurements were assessed three times and the final values were the mean from the data obtained. Body fat mass was estimated by bioelectrical impedance analysis (TBF-410, TANITA, Tokyo, Japan). Pubertal status was determined using the Tanner stage. Venous blood samples were obtained by specialized trained nurses at the Hospital after an overnight fast. Glucose, insulin and lipid profiles were determined by

standard autoanalyzer techniques. Insulin resistance was calculated from the homeostasis model assessment of insulin resistance (HOMA-IR) and another index, HOMA-AD, dividing HOMA-IR by adiponectin [26]. Additionally, the lipid accumulation product (LAP) was calculated, which is based on a combination of waist circumference (WC) and fasting triglycerides, and serves as a simple index for lipid over-accumulation among adults [27]. Since there is no data for the pediatric population, we calculated this index for children according to normal values of triglycerides and WC by age and sex. ELISA was used to measure high sensitivity IL-6, leptin, adiponectin and CNTF, all from R&D Systems, Minneapolis, MN. Serum CT-1 concentration was determined by ELISA (sensitivity 20 pg/mL; inter- and intra-assay coefficient of variation 8.40%-3.36%) as previously described with minor modifications [13]. Briefly, serum samples were added in triplicates to 96-well plates precoated with rat polyclonal anti-mouse CT-1 antibody (R&D Systems) and blocked in 0.1% Casein/PBS. Then a sandwich complex was formed with a polyclonal CT-1 antibody, developed in our laboratory in New Zealand White rabbits [28] labeled with biotin. After incubation, the unbound material was washed off, and the streptavidin-peroxidase complex (Pierce, Illinois) was added for detection of the bound CT-1. Subsequently, plates were washed, and antibody binding was determined using 3,3,5,5-tetramethylbenzidine substrate. Absorbance was read at 450 nm and the concentration was determined by comparison with serial dilutions of recombinant human CT-1.

2.4. Definition and components of the metabolic syndrome

We defined the MetS following the International Diabetes Federation definition (IDF) [29] for children aged 6-16 yr. According to this definition, the diagnosis of the MetS in children needs the presence of three risk factor components: WC above the 90th percentile, plus any 2 of the following factors: either systolic or diastolic blood pressure

≥130 mmHg or ≥85 mmHg respectively, or treatment with antihypertensive medication, triglyceride levels ≥150 mg/dL, HDL-cholesterol levels <40 mg/dL, and fasting plasma glucose levels ≥100 mg/dL or treatment for diabetes. At baseline and after the intervention the MetS criteria were applied to participants and the change in MetS components following the intervention was estimated.

2.5. Statistical analysis

Statistical analyses were performed using SPSS for Windows 15.0 software (SPSS Inc., Chicago, IL). $P < 0.05$ was considered statistically significant. The Shapiro-Wilk test was used to determine variable distributions. The sample size calculation indicated that we needed 20 subjects. This estimation was based on the following assumptions: an alpha error of 5%, a power of 90%, and a mean difference of 42±9 units in serum CT-1 levels after the intervention.

To assess the differences of parametric values before treatment between groups, the unpaired *t*-test was performed. We contrasted before *vs.* after intervention in subjects distributed by response, using the paired *t*-test. In addition, partial correlation coefficients adjusted for Tanner stage were performed to analyze the association between the change in CT-1 levels and changes in others variables, such as total cholesterol and WC. We also fitted multivariable linear regression analyses to estimate different associations of serum CT-1 levels with anthropometric and metabolic parameters, adjusting for potential confounders (Tanner stage and baseline CT-1) and obtaining β coefficients and p-values. Finally, an analysis of covariance (ANCOVA) was performed to assess changes in CT-1 levels according to the change in the number of MetS components after the 10-week intervention program, adjusting for Tanner stage and baseline CT-1.

3. RESULTS

At baseline, 91% of children who completed the 10-week WL program (22 boys and 22 girls, mean age 11.5 yr) were obese and one third was at risk of developing MetS. Characteristics of overweight/obese children according to the intervention response (the HR group who lost >0.50 BMI-SDS and the LR group when they lost ≤0.50 BMI-SDS of their initial BMI-SDS) are shown in Table 1. No differences were found between the two groups at baseline.

Both groups (HR and LR) after the intervention did significantly ($p<0.05$) decrease BMI-SDS, body fat, WC and serum glucose levels. After the 10-week intervention program, anthropometric and biochemical measurements significantly diminished in the HR group (Table 1). Mean WL was 3.73±0.24 kg, which corresponds to a 5.5% of the initial body weight ($p<0.001$) in the HR group. Specifically, they showed a significant reduction in BMI (-2 kg/m^2, $p<0.001$), BMI-SDS (-0.78, $p<0.001$), body fat mass (-8%, $p<0.001$), WC (-5.4 cm, $p<0.001$) and heart rate (-7.6 bpm, $p=0.028$) after the WL program. The rapid losses of weight and body fat during the intervention were associated with a marked improvement in glucose and lipid metabolism. Serum glucose (-6 mg/dL, $p<0.001$), total cholesterol (-0.54 mmol/L, $p<0.001$), LDL-cholesterol (-0.34 mmol/L, $p=0.003$), LAP (-9.84, $p=0.009$), insulin levels (-4.53 μU/mL, $p=0.010$) and HOMA-IR (-1.23, $p=0.003$) were significantly diminished. Notably, there was a decrease in serum CT-1 (-48 fmol/mL, $p=0.043$) levels in the HR group, while adiponectin levels were increased in these subjects (+1.03 μg/mL, $p=0.012$). No changes were observed in leptin, CNTF or IL-6 serum levels after the WL program in our population of overweight/obese children (Table 1).

Positive correlations were found between the relative (%) change in serum CT-1 levels and the change in WC ($r=0.432$, $p=0.050$) or total cholesterol levels ($r=0.611$, $p=0.003$) after adjusting for Tanner stage, but only in the HR group (Figure 1). No significant

correlations were observed in the LR group (data not shown). Moreover, in a multivariable linear regression, the relative (%) change in CT-1 serum levels significantly predicted the 41% and 28% of the variance in changes in total cholesterol and LDL-cholesterol, respectively, in the HR group independently of Tanner stage and baseline CT-1, while no associations were found in the LR group (Table 2).

The prevalence of MetS following the IDF criteria (as explained in the Subjects and Methods section) in the complete sample of overweight/obese children was 20% after the intervention (data not shown). When calculating the change in the MetS criteria after the WL program, we found subjects who decreased, maintained or either increased the number of MetS components, as shown in Figure 2. Interestingly, the lower the MetS risk factors, the lower the CT-1 levels after the 10-week nutritional intervention, (p ANCOVA=0.040, p trend=0.024).

4. DISCUSSION

This trial showed, for the first time, a decrease in serum CT-1 levels after a WL program in overweight/obese children and notably, that these diminished levels were strongly associated with a lower risk of developing the MetS.

The increased obesity prevalence in children is causing a severe problem in childhood and later in life, because obesity is associated with many comorbidities such as hypertension, dyslipidemia and disturbed glucose metabolism [30,31]. According to evidence, what seems to be the most successful approach against obesity is intensive lifestyle modification that includes guidance on dietary aspects, food-related factors, physical activity, and behavioral strategies [32-34]. Moreover, family support is of utmost importance. In this sense, in our study these comorbidities were improved after a 10-week nutritional intervention program that involved a modification of lifestyle habits.

Interestingly, overweight/obese children (HR and LR) had significantly reduced their BMI, BMI-SDS, body fat, WC and serum glucose levels after the intervention. Although, the LR group only maintained their body weight and showed no change in other parameters (such as CT-1). It is worthy to mention that overweight/obese children in our study were advised to follow a diet with a moderate calorie-restriction, so as not to interfere with their body growth. With regards to this, from a pediatrician's perspective, it is favorable to have no increase in body weight, especially fat mass, during a nutritional intervention to treat obese children [35].

In a recent meta-analysis of 899 obese children, a significant pooled BMI reduction of 1.25 kg/m^2 [5] was reported, similar to the BMI change achieved in our overweight/obese children (2.0 kg/m^2). A comparable observation was obtained for the percentage of body fat change in the former meta-analysis, with the lifestyle intervention group losing more than 3.2% of body fat than the usual care group [5]. Meanwhile, our overweight/obese children did show a higher reduction in body fat mass (-8%) after the WL program.

Nevertheless, it is reported that body weight reductions (between 0.25-0.50 BMI-SDS) can trigger an improvement in metabolic outcomes in obese children [6,35-37]. In addition, it is seen that the degree of WL was associated with an improvement in the prevalence of MetS and its components [38], as confirmed by our study.

Participants from the HR group showed increased adiponectin levels as expected [10]. Fasting glucose, insulin and insulin resistance (reduced HOMA-AD ratio and HOMA-IR) were successfully reduced after the nutritional intervention, as reported elsewhere [11,12,39]. Similar to our work, important decreases in heart rate, total cholesterol, LDL-cholesterol and triglycerides were achieved after short term interventions in obese children [11,12,40,41].

However, we found no change in blood pressure, CNTF, leptin, and IL-6 serum levels in the HR group. CNTF is a pluripotent neurocytokine that mimics the biological actions of leptin overcoming "leptin resistance" [42], but no information on CNTF levels after a WL program is available. In our overweight/obese children circulating leptin did not change after the intervention, as shown by Cambuli *et al*. [10] in similar aged obese children. Moreover, it is reported that leptin concentration exhibits significant changes during progressive pubertal stages [43].

Furthermore, decreased levels or no change - as seen in our study for IL-6- were observed in obese children following WL programs [44-46]. The reported differences could be explained by the intervention design (duration, type, and lifestyle changes), characteristics of participants (age, sex, family lifestyle) and other factors.

Strengths of our study include: 1) measurements in young subjects not confounded by chronic obesity-related disorders; 2) the overweight/obese subjects, especially HR, achieved substantial WL in a short term dietary intervention showing a good response to the diet (WL higher than 5% of initial body weight) [47,48] and 3) a standardized intervention with similar dietary conditions applied to a relatively homogeneous group. On the other hand, weaknesses of the study are: 1) the lack of the comparison group of normal-weight children; 2) the small number of children per group; and 3) a large number of participants were in progressive pubertal stages with a high rate of growth and significant endocrine changes.

Interestingly, our study showed for the first time a significant decreased in CT-1 levels after a WL program in the HR overweight/obese children. Our results are in line with those reported for other cytokines such as leptin, IL-6 [44], or visfatin [49] in WL intervention studies with obese children from similar age ranges. Although, CT-1 is a

protective cytokine, Moreno-Aliaga *et al.* suggested that it could be increased in obesity as a compensatory mechanism to fight against this disease [13].

Nevertheless, the change in CT-1 levels strongly predicted the 41% and 28% of the variance in changes in total cholesterol and LDL-cholesterol, respectively, during rapid WL in our overweight/obese children. Likely, Murer *et al*. [50] did show that changes in leptin levels were predictors for cholesterol and LDL-cholesterol after a WL program in obese children and adolescents [50]. Our results indicate that the greater the reduction in CT-1 levels, the greater the reduction in cholesterol levels, confirming the potential role of CT-1 in ameliorating unfavorable metabolic features.

Moreover, we were able to show for the first time a relationship between CT-1 levels and MetS risk factors in overweight/obese children. Specifically, we found that the lower the CT-1 levels after the intervention, the higher the reduction in the risk of developing MetS.

5. CONCLUSION

Our study showed for the first time a decrease in serum CT-1 levels after a WL program, and these diminished CT-1 levels were strongly associated with a reduction in cholesterol levels and in the risk of developing MetS in overweight/obese children. Consequently, our data may suggest that CT-1 could be an indirect marker for the diagnosis of MetS in this population.

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AUTHOR CONTRIBUTIONS

Amelia Marti, J. Alfredo Martínez, Matilde Bustos and María Jesús Moreno-Aliaga were involved in the design, funding and writing of the manuscript. María Chueca, Mirentxu Oyarzabal and María Cristina Azcona-Sanjulián were involved in the design and conducting of the manuscript. Tara Rendo-Urteaga, Sonia García-Calzón and Eduardo Martínez-Ansó were involved in the conducting, analysis and writing of the manuscript. The authors have no competing interests.

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Table 1: Characteristics of overweight/obese children before and after 10-week of treatment according to the response.

	High responders (n=22)			Low responders (n=22)			
	Baseline	After intervention	p value[1]	Baseline	After intervention	p value[1]	p value[2]
Sex (boys/girls, n)	14/8			8/14			0.073
Tanner stage (I/II/III, n)	7/12/3			4/16/2			0.618
Age (y)	11.23 ± 0.59			11.82 ± 0.52			0.458
Weight (Kg)	68.16 ± 3.66	64.43 ± 3.58	<0.001	77.1 ± 4.87	76.46 ± 4.97	0.074	0.149
Height (m)	1.51 ± 0.03	1.52 ± 0.03	<0.001	1.56 ± 0.03	1.56 ± 0.03	0.001	0.252
Body fat (%)	35.59 ± 1.77	32.64 ± 1.55	<0.001	39.00 ± 1.58	38.21 ± 1.52	0.024	0.157
BMI (kg/m^2)	29.33 ± 0.85	27.33 ± 0.86	<0.001	31.18 ± 1.22	30.60 ± 1.26	0.001	0.221
BMI-SDS	4.01 ± 0.39	3.23 ± 0.36	<0.001	3.99 ± 0.49	3.79 ± 0.50	<0.001	0.975
Waist circumference (cm)	89.89 ± 2.11	84.49 ± 2.00	<0.001	92.34 ± 1.96	90.06 ± 2.14	0.006	0.400
Hip circumference (cm)	98.50 ± 2.23	95.23 ± 2.22	<0.001	104.58 ± 2.93	103.72 ± 3.21	0.205	0.106

Waist to hip ratio	0.91 ± 0.01	0.89 ± 0.01	<0.001	0.89 ± 0.01	0.87 ± 0.01	0.054	0.175
Waist to height ratio	0.59 ± 0.01	0.56 ± 0.01	<0.001	0.59 ± 0.01	0.58 ± 0.01	0.002	0.967
Systolic BP (mmHg)	127.95 ± 4.39	125.80 ± 3.28	0.695	131.05 ± 3.39	125.67 ± 3.40	0.211	0.578
Diastolic BP (mmHg)	75.40 ± 3.25	70.10 ± 2.44	0.259	71.62 ± 3.94	67.67 ± 1.93	0.281	0.466
Heart rate (bpm)	83.63 ± 2.85	76.05 ± 2.39	0.028	80.42 ± 2.55	78.58 ± 4.47	0.549	0.547
Glucose (mg/dL)	93.00 ± 1.74	87.00 ± 1.71	<0.001	91.50 ± 1.04	85.68 ± 1.62	0.003	0.464
Insulin (μU/mL)	17.92 ± 1.63	13.39 ± 1.36	0.010	17.95 ± 2.13	18.57 ± 2.73	0.649	0.992
HOMA-IR	4.13 ± 0.38	2.90 ± 0.31	0.003	4.07 ± 0.49	3.99 ± 0.60	0.822	0.929
HOMA-AD ratio	344.63 ± 91.07	223.66 ± 77.69	<0.001	339.16 ± 54.09	353.76 ± 66.47	0.604	0.453
Total cholesterol (mmol/L)	4.61 ± 0.20	4.07 ± 0.18	<0.001	4.34 ± 0.15	4.33 ± 0.19	0.898	0.299
HDL cholesterol (mmol/L)	1.33 ± 0.07	1.21 ± 0.06	0.001	1.23 ± 0.06	1.16 ± 0.05	0.005	0.415
LDL cholesterol (mmol/L)	2.73 ± 0.16	2.39 ± 0.14	0.003	2.51 ± 0.12	2.54 ± 0.16	0.798	0.329
Triglycerides (mmol/L)	0.99 ± 0.09	0.80 ± 0.08	0.050	1.23 ± 0.16	1.24 ± 0.18	0.991	0.184
Lipid Accumulation Product (LAP)	33.98 ± 3.53	24.14 ± 3.91	0.009	44.98 ± 5.94	44.52 ± 8.34	0.938	0.120
Ratio total cholesterol/HDL cholesterol	3.64 ± 0.21	3.55 ± 0.22	0.457	3.70 ± 0.23	3.85 ± 0.20	0.159	0.883

Leptin (ng/mL)	26.95 ± 3.26	46.62 ± 6.86	0.050	36.62 ± 4.47	34.76 ± 4.84	0.693	0.095
CNTF (pg/mL)	24.98 ± 0.19	24.94 ± 0.17	0.823	25.01 ± 0.17	25.09 ± 0.16	0.598	0.919
Adiponectin (µg/mL)	7.62 ± 0.90	8.65 ± 1.04	0.012	6.03 ± 0.48	5.51 ± 0.36	0.130	0.101
IL-6 (pg/mL)	2.13 ± 0.22	1.88 ± 0.26	0.404	1.84 ± 0.20	2.36 ± 0.37	0.072	0.327
CT-1 (fmol/mL)	653.58 ± 89.05	605.58 ± 83.50	0.043	461.38 ± 78.23	452.85 ± 62.53	0.837	0.114

Variables are expressed as mean ± SEM. BMI-SDS: Standard Deviation Score for BMI; BP: Blood Pressure; HOMA-IR: Homeostasis Model Assessment of Insulin Resistance; HOMA-AD: HOMA to adiponectin ratio; CNTF: Ciliary Neurotrophic Factor; IL-6: Interleukin-6; CT-1: Cardiotrophin-1.

1: p value for the comparison between before and after intervention in subjects distributed by the response.

2: p value for the comparison at baseline between high and low responders.

2

3

Table 2: Association between the percentage of change of CT-1, after the 10-week intervention, with changes in anthropometric and metabolic measures in the high and low responder groups of overweight/obese children: multivariable linear regression analyses.

		% Δ WC		% Δ Total Cholesterol		% Δ LDL cholesterol		% Δ HOMA-IR	
		β	p	β	p	β	p	β	p
High responders (n=22)	% Δ CT-1[1]	0.430	0.046	0.619	0.002	0.520	0.016	-0.239	0.285
	% Δ CT-1[2]	0.428	0.057	0.648	0.003	0.532	0.023	-0.280	0.265
Low responders (n=22)	% Δ CT-1[1]	0.265	0.246	0.078	0.738	0.149	0.520	-0.149	0.520
	% Δ CT-1[2]	0.293	0.201	0.074	0.753	0.171	0.458	-0.120	0.606

CT-1: Cardiotrophin-1; WC: Waist Circumference; HOMA-IR: Homeostasis Model Assessment of Insulin Resistance.

1 Crude standardized regression value, n=22.

2 Adjusted for Tanner stage and baseline CT-1.

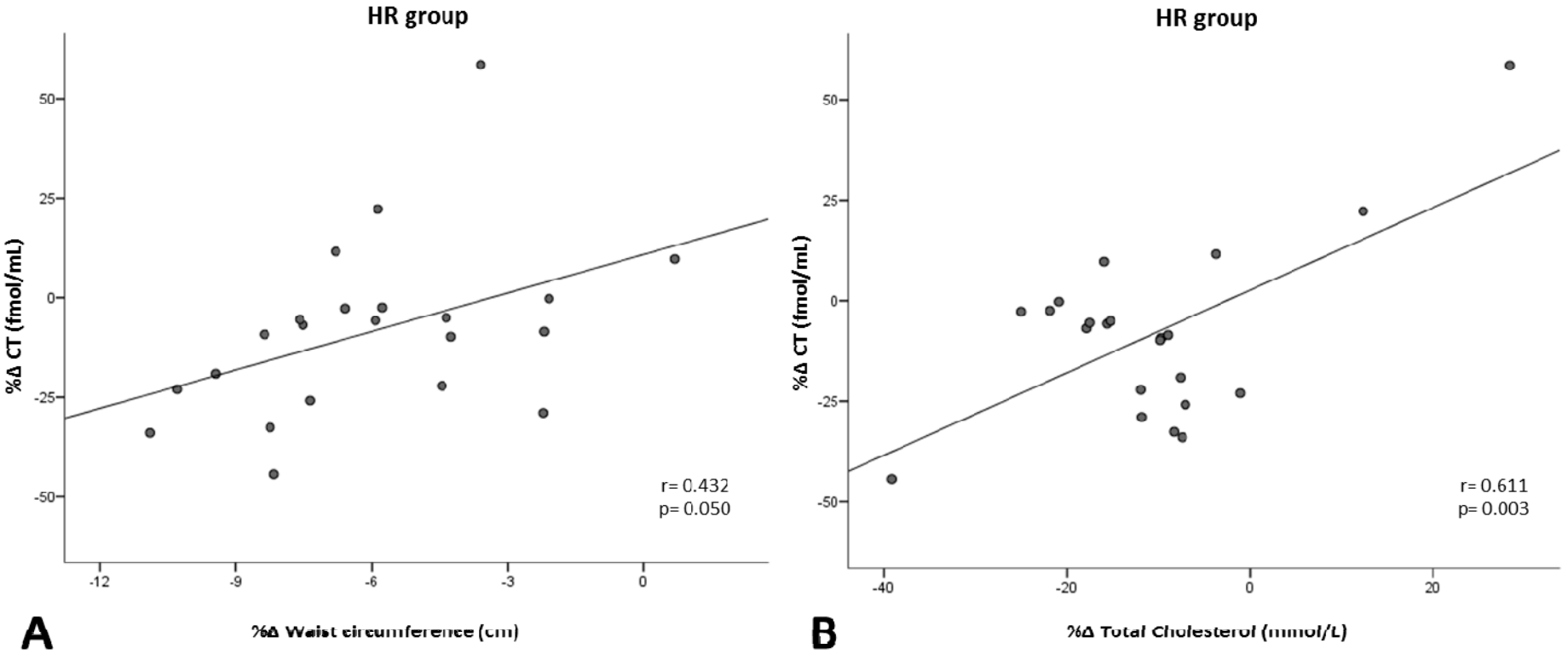

Figure 1: Association between the change in CT-1 levels (%) and in waist circumference or total cholesterol levels after the intervention. Positive correlations were found in the HR group (A,B), after adjusting for Tanner stage.

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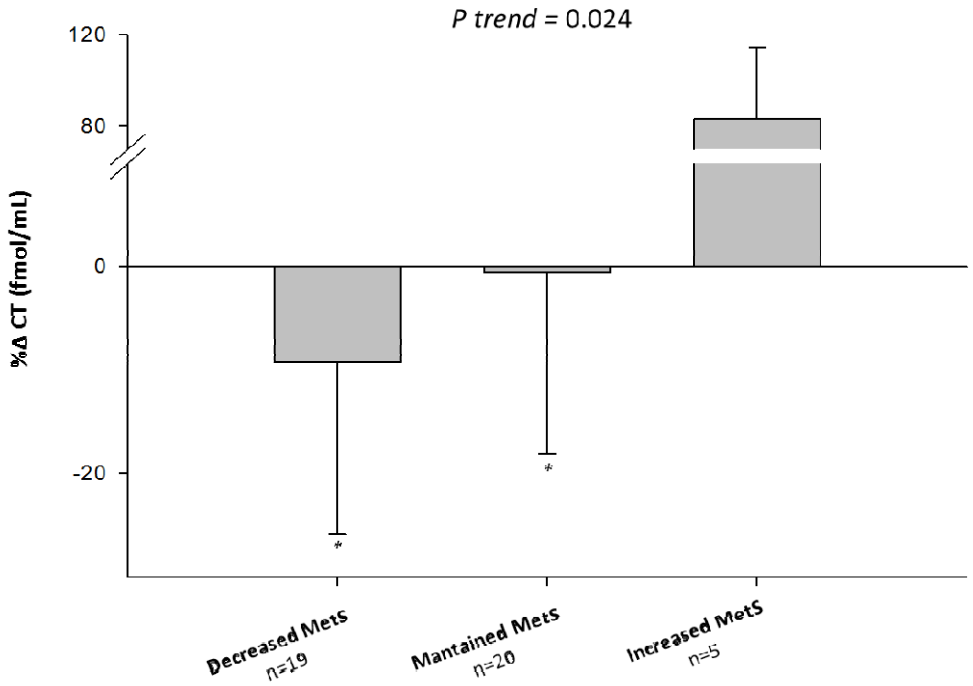
P trend = 0.024
120
80
0
-20
%Δ CT (fmol/mL)
*
*
Decreased MetS
n=19
Mantained MetS
n=20
Increased MetS
n=5

15

Figure 2: The change in serum CT-1 levels (%) after the 10-week intervention, according to the change in MetS risk components. Between group comparison for the three groups: *p* (ANCOVA) = 0.040 after adjusting for Tanner stage and baseline CT-1. *: $p<0.05$ between participants who decreased or maintained *vs.* increased MetS risk components.