Vitamin C Restores Blood Pressure and Vasodilator Response During Mental Stress In Obese Children

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Abstract

Background: Peripheral vasodilation response plays an important role in the pathophysiology of obesity and heart disease.

Objective: To evaluate the chronic effect of vitamin C (VitC) supplementation on blood pressure and on vasodilation response to mental stress.

Methods: In a double-blind, randomized and prospective study we evaluated obese children with 8 to 12 years in 2 similar groups: 1) supplemented with 500 mg VitC (n = 11) and 2) placebo (n = 10) for 45 days. Eight age-matched lean control children were also studied. We evaluated: mean blood pressure (MBP), heart rate (HR) and forearm blood flow by venous occlusion plethismography. Forearm vascular conductance (FVC) was calculated by: (forearm blood flow/PAM) X100.

Results: On pre-intervention evaluations obese children showed higher MBP and lower FVC compared to lean control children. After intervention VitC diminished MBP at rest (81 ± 2 *vs* 75 ± 1 mmHg, p = 0.01), whereas placebo did not promote changes in MBP (p = 0.58). In addition, VitC promoted FVC increase at rest (3.40 ± 0.5 *vs* 5.09 ± 0.6 un, p = 0.04) and during the mental stress (3.92 ± 0.5 *vs* 6.68 ± 0.9 un, p = 0.03). Moreover, after VitC supplementation FVC levels were similar to the lean control children at rest (5.09 ± 0.6 *vs* 5.82 ± 0.4 un, p > 0.05) and during mental stress (6.68 ± 0.9 *vs* 7.35 ± 0.5 un, p > 0.05).

Conclusion: VitC supplementation reduced the MBP and restored peripheral vasodilatation response during mental stress in obese children. (Arq Bras Cardiol. 2011; [online].ahead print, PP.0-0)

Keywords: Ascorbic acid; blood pressure; obesity; child; stress, psychological.

Introduction

The prevalence of obesity has grown significant around the world, and this phenomenon has been observed in developing or industrialized countries[1]. Even more worrying is the fact that the prevalence of obesity is also increasing rapidly in children and adolescents, reaching numbers in excess of 10% in these countries[2,3].

Accumulating evidence has improved the understanding of the implications of obesity on the cardiovascular system and its regulatory mechanisms. Obese people present baroreflex dysfunction[4], increased peripheral vascular resistance[5], high oxidative stress[6], as well as increased cardiac and muscular sympathetic activity[7,8]. These changes may lead to increased levels of blood pressure and decreased blood flow to muscles. During sympathoexcitatory maneuvers, such as isometric exercise, cold pressor test or mental stress, the vasodilator reflex that should increase muscle blood flow is attenuated in obesity[5,9]. Furthermore, these changes are already present in obese children and it has been shown that non-pharmacological therapy based on diet and exercise can restore the physiological responses of blood pressure and vasodilation during physiological maneuvers of mental stress and isometric exercise[10,11].

In normotensive and strophic individuals, mental stress causes vasodilatation reflex in the forearm, which has largely been attributed to nitric oxide production by stimulation of adrenergic receptors b2 and muscarinic receptors[12]. Previous studies have shown that treatment with antioxidants such as vitamin C in high doses act primarily by reducing superoxide anions, while chronic oral therapy would increase nitric oxide production and/or activate the action of antioxidants, restoring endothelial function in patients with cardiovascular diseases and obese individuals[6,13].

The use of vitamin C could be a therapeutic or associative option to treat hemodynamic changes in childhood obesity. Thus, to assess the reversibility of the injury in early vasodilator function in obese children, we have studied a randomized intervention protocol for vitamin C. The purpose of this study was to test the hypothesis that vitamin C, when chronically

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administered at high doses, can restore muscle vasodilator response in the forearm during mental stress in obese children.

Methodology

Case series

Twenty-one obese children were selected from the Clinic of Endocrinology, University Hospital, Federal University of Paraíba. These children met the following criteria for inclusion/exclusion: (1) age between 8 and 12 years; (2) both genders; (3) body mass index (BMI) > 97%, BMI z-score, 2.8 to 4.3, (4) not under medication; (5) no evidence of cardiovascular, respiratory, hormonal or metabolic disease during the study; and (6) no systematic regular physical activity. After selection, we obtained the consent form signed by the legal guardians. In this double-blind, randomized prospective study, these children were divided randomly, using the Research Randomizer website - www.randomizer.org into two groups: 1) group of children supplemented with 500 mg of vitamin C (n = 11) and 2) placebo substance (n = 10) for 45 days. Obesity was defined using the Z-Score for specific BMI for age and gender[14]. Eight normal-weight children matched for age were also enrolled in the study (BMI between 16 and 19 kg/m^2). The study was approved by the Ethics Committee on Human Research of the Federal University of Paraíba (CEP/CCS) protocol # 0466-2009.

Measurements and procedures

Anthropometric measurements

Body weight was measured by electronic weight scale with children dressed in light clothing (shorts and T-shirt). Height was measured by Harpenden stadiometer.

Blood pressure, heart and respiratory rates

Blood pressure was monitored non-invasively and intermittently through an automated oscillometric device (Dixtal™, DX 2020, Manaus, Brazil). The occluder cuff was placed around the right ankle of the child; then it was inflated automatically every 30 seconds. Heart rate was monitored continuously in lead II of the electrocardiogram and respiratory rate was obtained through the respiratory belt, which contains bilateral sensors that capture the respiratory signal by thoracic distensibility. The signals were acquisitive, with the aid of software WINDAQ/DATAQ DI200 with a sampling frequency of 1000 Hz per channel.

Blood flow and forearm vascular conductance

Blood flow was assessed by the technique of venous occlusion plethysmography. A silastic tube filled with mercury connected to a low pressure transducer was placed around the forearm 5 cm distally from the humeral-radial articulation and connected to the plethysmograph. A cuff was placed around the wrist and another on the upper arm. The wrist cuff was inflated to a suprasystolic level one minute before starting the measurements. At intervals of 10 seconds, the arm cuff was inflated above venous pressure for 7 to 8 seconds. The increased tension in the silastic tube reflected the increased volume of the forearm and thus vasodilation. Muscle flow wave signal was acquired online through the program WINDAQ DI200, at a frequency of 1000 Hz. The forearm vascular conductance was calculated as the ratio of blood flow in the forearm (ml/min/100 ml) and mean arterial pressure (mmHg).

Mental stress test

The mental stress test was performed using the Stroop color-word test[15]. During the Stroop color-word test, children were shown a series of color names printed in a different color from the name of the color presented. They were asked to identify the color of the print rather than reading the word.

Blood biochemistry

Blood samples were collected to determine concentrations of cholesterol and subfractions (LDL and HDL), triglycerides and blood glucose. Enzymatic calorimetric tests were used to analyze cholesterol and subfractions and glucose levels.

Experimental protocol

Baseline measurements of blood flow in the forearm, mean arterial pressure, heart rate and respiratory rate were recorded for 3 minutes. The mental stress was conducted for 3 minutes, with simultaneous recording of blood flow in the forearm, mean arterial pressure, heart rate and respiratory rate. The assessment of the degree of difficulty of the test was performed at the end of the application, using a standard five–point scale: 0 - not stressful; 1 - little stressful; 2 - stressful; 3 - very stressful; and 4 - very, very stressful.

Statistical analysis

To test the normal distribution of data we used the Kolmogorov-Smirnov test. Possible differences between groups were tested by Student's t-test for independent samples. Two-way ANOVA for repeated measures was used to test differences within groups and between groups vitamin C and placebo. In addition, two-way ANOVA was used to test differences within groups and between groups during mental stress. When significant differences were found, comparison with Scheffé post-hoc test was performed. Data were presented as mean ± standard error of the mean, accepting $p < 0.05$ as significance level.

Results

Effect of obesity

Baseline data

The anthropometric and hemodynamic characteristics in obese and normal weight children at baseline conditions are shown in Table 1. The variables age and height are similar in obese and eutrophic children. Body weight and BMI were significantly higher in obese children. Comparing hemodynamic variables, we observe that the values of heart rate were not statistically different between groups. In turn, mean arterial pressure was higher in obese children. In addition, blood flow and forearm vascular conductance were lower in obese children when compared to normal weight.

Mental stress response

During mental stress, there was no statistical difference between the levels of perception informed by obese and eutrophic children after the test (1.85 ± 0.34 *vs* 2.5 ± 0.18, $p = 0.15$). This conclusion ensures that perception during mental stress test was similar to the groups studied.

Information of heart rate and blood pressure in obese and eutrophic children is shown in Table 2. Heart rate increased significantly during mental stress in both groups ($p < 0.05$) during mental stress. However, when we made the comparison between the groups, no significant differences were found. Mean arterial pressure also increased significantly during mental stress in the groups ($p < 0.05$). Additionally, a comparison between groups showed that mean blood pressure was significantly higher in obese children ($p < 0.05$).

During mental stress, blood flow increased significantly from the first minute of mental stress in the group of normal-weight children, whereas in the group of obese children, there was increase only in comparison to rest in the final minute of mental stress ($p < 0.05$ - Table 1). Forearm vascular conductance increased significantly in the group of normal-weight children, but not in the group of obese children ($p < 0.05$ - Fig. 1). Moreover, comparison between groups showed that the values of blood flow (Table 2) and forearm vascular conductance were significantly higher in the group of normal-weight children from rest until the 3rd minute of mental stress ($p < 0\ 05$ - Fig. 1).

Effect of intervention with vitamin C or placebo

Baseline data

Anthropometric and metabolic characteristics before and after intervention with vitamin C or placebo are presented in Table 3. Age, weight, height, BMI and metabolic variables were similar in obese children randomized for vitamin C or placebo.

Table 1 - **Anthropometric and hemodynamic characteristics in obese and eutrophic children at rest**

	Obese (n = 21)	Eutrophic (n = 8)	p
Age (years)	10.9 ± 0.4	10.2 ± 0.7	0.80
Height, m	1.49 ± 0.2	1.40 ± 0.1	0.38
Weight, kg	64.3 ± 4.1	33.3 ± 2.4	0.01
BMI, kg/cm²	29 ± 1	18 ± 1	0.01
Z-score BMI	3.8 ±0.2	0.8 ± 0.1	0.01
HR, bpm	79 ± 3	76 ± 2	0.22
MBP, mmHg	79 ± 1	71 ± 1	0.01
FABF, ml.min^{-1}.100g^{-1}	2.85 ± 0.2	4.15 ± 0.1	0.01
FAVC, units	3.88 ± 0.3	4.63 ± 0.5	0.03

BMI - body mass index; HR - heart rate; MAP - mean arterial pressure; FABF - forearm blood flow; FAVC - forearm vascular conductance. Values in mean ± standard error.

Table 2 - **Hemodynamic responses in obese and eutrophic children during mental stress**

	Rest	Mental stress		
		1 min	2 min	3 min
HR, bpm				
Obese	79 ± 3	82 ± 2	84 ± 2†	85 ± 3†
Eutrophic	76 ± 2	82 ± 2†	86 ± 3†	87 ± 4†
MBP, mmHg				
Obese	79 ± 1*	86 ± 3*†	91 ± 3*†	96 ± 3*†
Eutrophic	71 ± 1	78 ± 5	84 ± 5†	86 ± 4†
FSA, ml.min^{-1}.100g^{-1}				
Obese	2.85 ± 0.2*	3.20 ± 0.3*	3.50 ± 0.3*	3.88 ± 0.3*†
Eutrophic	4.15 ± 0.1	5.03 ± 0.2†	5.39 ± 0.2†	5.77 ± 0.1†

*HR - heart rate; MAP - mean arterial pressure; FABF - forearm blood flow. Values in mean ± standard error. * p < 0.05,* vs *eutrophic, † p < 0.05,* vs *rest.*

Intervention with vitamin C or placebo did not cause significant changes in total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides or glucose in obese children during the period studied.

Mental stress response

The values of heart rate, mean arterial pressure and blood flow in the forearm, during rest and mental stress, pre and post-intervention with vitamin C or placebo in obese children are shown in Table 4.

Vitamin C or placebo did not cause significant changes in the values of heart rate at rest, or during mental stress. Mean arterial pressure decreased significantly after vitamin C, at rest and during mental stress; however, this event was not observed after supplementation with placebo. Comparing the situation pre with post-intervention, vitamin C increased blood flow and forearm vascular conductance (Fig. 2) significantly both at rest and during mental stress. This same finding was not observed after intervention with placebo substance (Fig. 3). Interestingly, the major finding of this study was the fact that increased blood flow and vascular conductance in the forearm was so important that vasodilation at rest and during mental stress reached values close to those observed in the group of normal-weight children.

Discussion

This randomized, double-blind study showed that in obese children: 1) the response of mean arterial pressure during mental stress is exacerbated; 2) the muscle vasodilator response in the forearm during mental stress is decreased; 3) vitamin C reduced the mean arterial pressure during mental stress to normal levels; and 4) vitamin C regularized the muscular vasodilatory response during mental stress.

The novelty of this study refers to the administration of vitamin C. Our results demonstrate that in obese children, this therapy can lead to a reduction in blood pressure and increase muscle blood flow, both at rest and in response to challenges such as mental stress.

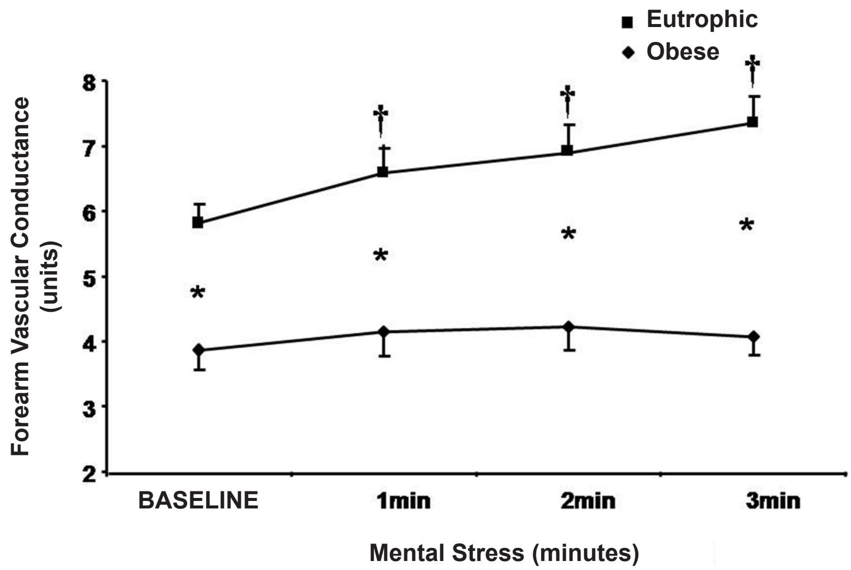

*Figure 1 - Absolute values of forearm vascular conductance during mental stress in obese and eutrophic children. Observe that vasodilation is impaired in the group of obese children. * p < 0.05 comparison between groups; † p < 0.05 vs rest.*

Table 3 - **Effect of interventions on anthropometric and metabolic characteristics in obese children**

	Vitamin C (n = 11)		Placebo (n = 10)	
	Pre	Post	Pre	Post
Age (years)	11.2 ± 0.4		10.8 ± 0.4	
Weight, kg	64.3 ± 2.3	60.1 ± 5.2	64.3 ± 2.1	62.4 ± 3.2
Height, m	1.40 ± 0.9		1.49 ± 0.2	
BMI, kg/cm²	29 ± 1	26 ± 2	28 ± 2	27 ± 1
Z-score BMI	3.9 ± 0.2	3.7 ± 0.2	3.8 ± 0.2	3.7 ± 0.2
Total cholesterol, mg/dl	160 ± 17	158 ± 10	167 ± 15	167 ± 5
HDL cholesterol, mg/dl	44 ± 5	44 ± 1	49 ± 11	49 ± 10
LDL cholesterol, mg/dl	91 ± 10	89 ± 12	90 ± 10	90 ± 10
Triglycerides, mg/dl	110 ± 18	105 ± 9	99 ± 14	100 ± 7
Glucose, mg/dl	80 ± 8	82 ± 10	90 ± 3	89 ± 5

BMI - body mass index; HDL - high intensity lipoprotein; LDL - low intensity lipoprotein.

Impact of obesity on the hemodynamic response to mental stress

The relationship between obesity and increased blood pressure in obese children has been consistently published in the literature[10,16]. In our study, we confirmed that the blood pressure of obese children at rest is significantly higher when compared to normal-weight children. In agreement with another study, we also observed that obese children have increased blood pressure response during sympathoexcitatory maneuvers, including mental stress[10]. We believe that increased blood pressure response during mental stress in obese children may be due to abnormal neurovascular control, since the existence of sympathetic overactivity, baroreflex dysfunction and muscular vasodilator dysfunction is already known[11,17]. In obese adults, sympathetic activation has been considered responsible for the increased peripheral vascular resistance and muscle as a result of pressure pressure[7]. Another possibility to explain an increased blood pressure could be by way of change in cardiac output. However, our findings do not support this hypothesis, since the heart rate response to mental stress was similar in obese and eutrophic children.

Reduced muscle blood flow and forearm vascular conductance have been well characterized in obese children, adolescents and adults[5,10,18]. Our findings are consistent with these findings in the literature, since we also found that the vascular conductance and blood flow in the forearm muscles

Table 4 - **Effect of hemodynamic response in obese and eutrophic children during mental stress**

	Rest	Mental stress		
		1 min	2 min	3 min
HR, bpm				
Vitamin C				
Pre	81 ± 3	82 ± 2	83 ± 2	87 ± 4†
Post	77 ± 4	81 ± 2	83 ± 3	82 ± 4†
Placebo				
Pre	79 ± 3	83 ± 2	81 ± 3	85 ± 4
Post	81 ± 4	82 ± 3	81 ± 2	85 ± 4
MBP, mmHg				
Vitamin C				
Pre	81 ± 2*	89 ± 2*†	92 ± 2*†	98 ± 2*†
Post	75 ± 1	82 ± 2†	86 ± 3†	88 ± 3†
Placebo				
Pre	76 ± 2	82 ± 4	92 ± 5†	94 ± 4†
Post	80 ± 3	85 ± 2	93 ± 4†	94 ± 3†
FABF, ml.min^{-1}100g^{-1}				
Vitamin C				
Pre	2.80 ± 0.4*	3.23 ± 0.4*	3.62 ± 0.4*	3.80 ± 0.5*†
Post	3.81 ± 0.4	5.09 ± 0.7†	5.55 ± 0.7†	5.83 ± 0.8†
Placebo				
Pre	2.97 ± 0.3	3.61 ± 0.4	3.80 ± 0.3†	3.98 ± 0.4†
Post	2.94 ± 0.3	3.17 ± 0.3	3.50 ± 0.5†	3.78 ± 0.6†

*HR - heart rate; MAP - mean arterial pressure; FABF, forearm blood flow. Values in mean ± standard error. * $p < 0.05$ vs eutrophic, † $p < 0.05$ vs rest.*

are decreased in obese children. Additionally, we found that vasodilation during mental stress is markedly decreased in obese children. This finding corroborates previous findings, whose vasodilator response during reactive hyperemia or isometric exercise is reduced in obese children[7,19]. These changes are worrisome, since it is already known that endothelial dysfunction of blood vessels in childhood obesity is an early event for atherogenesis and for the formation of markers of arterial injury, which precedes the formation of platelets[19,20].

Effect of supplementation with vitamin C in the hemodynamic response to mental stress

The purpose of this study was to test the hypothesis that vitamin C, when chronically administered at high doses, can restore muscle vasodilator response in the forearm during mental stress in obese children.

Vitamin C has been recognized as a potent vasodilator antioxidant and this effect has been suggested especially in randomized and cross-sectional studies[21-23]. To confirm the feasibility of using an antioxidant therapy in obese children in this study, we chose the use of vitamin C, since its action and safety are well documented in the literature.

Vitamin C is especially known for being a water-soluble antioxidant that removes superoxide anions and other oxygen reactive species. Studies with obese hypertensive patients or patients with heart failure[6,21,22] have shown that ascorbic acid levels are decreased in these groups when compared to control. There, we concluded that the lower concentration of ascorbic acid could have been caused by excessive oxidative stress[23]. In our study, it was not possible to complete this assessment, since the methodology employed was not sensitive enough to detect changes caused by chronic use of vitamin C. Yet,

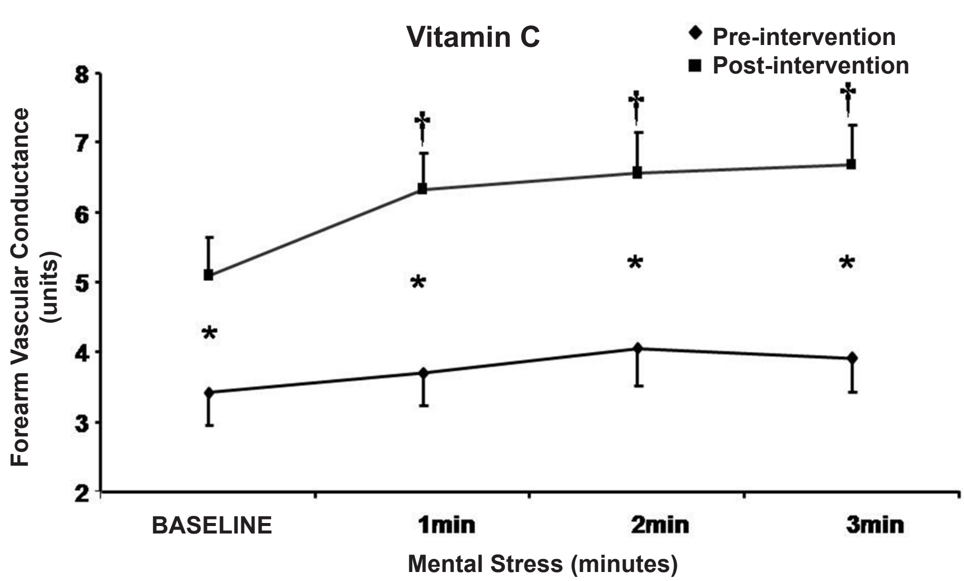

***Figure 2** - Absolute values of forearm vascular conductance during mental stress pre and post-intervention with vitamin C. Note that vasodilation is increased after intervention. * $p < 0.05$ comparison between pre and post-intervention; † $p < 0.05$, vs rest.*

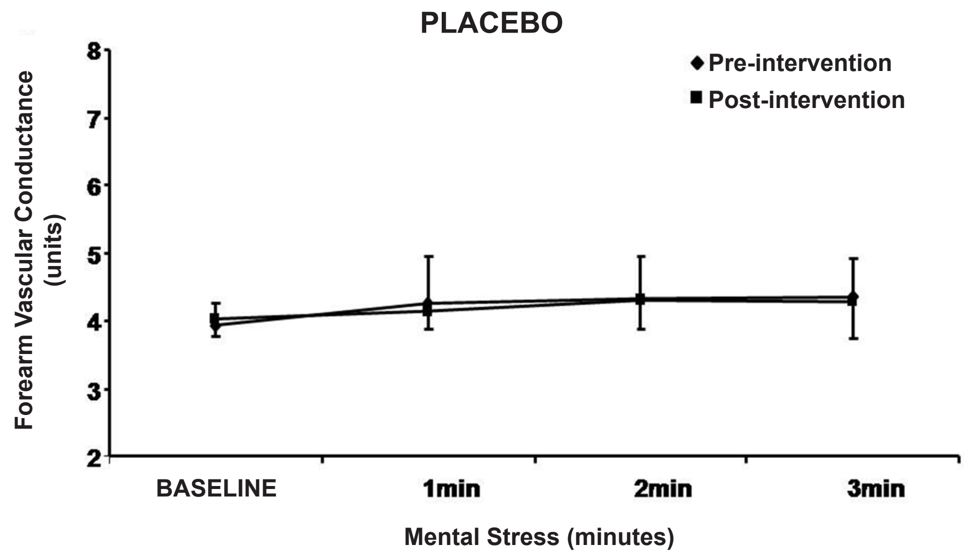

Figure 3 - Absolute values of forearm vascular conductance during mental stress pre and post-intervention with placebo substance.

we can conclude from our findings that oxidative stress has probably caused changes in blood flow and forearm vascular conductance in obese children, since the muscle vasodilator response was restored after supplementation with vitamin C[23,24].

Besides oxidative stress, other pathophysiological mechanisms are responsible for hemodynamic impairment of obesity such as diabetes, abnormalities in lipid metabolism, glucose and blood pressure. In our study, the effects of intervention with vitamin C were very important. Administration of vitamin C for 45 days significantly increased vasodilation in the forearm muscle at rest and during mental stress. This increase was amazing, and the values of blood flow and forearm vascular conductance have increased post-intervention and showed values similar to those observed in normal-weight children. The fact that vitamin C is an antioxidant able to restore impaired vasodilator function is not new; different authors have demonstrated that intravenous vitamin C restores the vasodilator response in different cardiovascular diseases[13,25]. These authors have speculated that one mechanism likely to explain the action of vitamin C in the restoration of vasodilator function is that it may be acting on the inactivation of free radicals, resulting in high oxidative stress, which are responsible for the reduced activity of vasoactive factors from the endothelium[26]. We attribute the positive changes in blood flow and vascular conductance to the inactivation of free radicals by the administration of Vitamin C, since there were no significant changes in body weight, BMI or on the metabolic profile, which could also be involved in improved vasodilation.

Another important result observed in this study was reported for blood pressure, in which vitamin C significantly reduced mean arterial pressure at rest and during mental stress in obese children. We have not evaluated systolic or blood volume, thus we cannot confirm whether this reduction in blood pressure was through change in cardiac output. However, we suggest that the reduction of mean arterial pressure could be associated with attenuation of sympathetic activity.

In conclusion, supplementation with vitamin C for 45 days normalizes the mean arterial pressure in obese children, at rest and in response to mental stress. Additionally, comparing the values of blood flow and forearm vascular conductance between the groups of obese children who received vitamin C and placebo substance, we notice that they were restored to near normal values during mental stress.

Limitations

We recognize some limitations in our study. It was not possible to quantitate oxidized LDL (a marker of oxidative stress) in obese children; however, we indicate the studies by Vincent and Taylor[27] and Block et al[28] showing that oxidative stress is increased in obesity. In our study, it was not possible to assess the concentration of ascorbic acid, and if so, we suggest the assessment of the study by Perticone et al[6]. To minimize this limitation, we studied the daily intake of vitamin C in obese children from the food frequency questionnaire proposed by Block et al[28]. In this evaluation, we have found average daily intake of vitamin C from 144 ± 19 mg.

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