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The effect of *Citrus Aurantifolia* (Lemon) peels on cardiometabolic risk factors and markers of endothelial function in adolescents with excess weight: A triple-masked randomized controlled trial

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Abstract

Background: Childhood obesity is becoming a global problem and its incidence is increasing. The role of dietary intervention with fruits containing vitamin C and flavonoid to control obesity consequences in childhood has not been yet defined. Lemon (*Citrus aurantifolia*) peels contain flavonoid, pectin and vitamin C. We aimed to compare the effects of lemon peels and placebo on cardiometabolic risk factors and markers of endothelial function among adolescents with overweight and obesity.

Methods: In this triple-masked, randomized controlled trial, 60 overweight/obese adolescents were enrolled in a 4-week trial. Eligible participants were randomly assigned into two groups of equal number receiving daily oral capsules containing lemon powder or placebo. Fasting blood sugar, lipid profile, ICAM-1 and VCAM-1, as well as systolic and diastolic blood pressure were compared between the two groups before and after administration of medication and placebo.

Results: Of the total 60 enrolled patients, 30 and 29 patients in the lemon and control groups completed the study, respectively. The results of within-group analysis demonstrated a slight reduction in body mass index, LDL-C and systolic blood pressure in the lemon group, but no between group differences existed in the studied variables.

Conclusion: This study revealed that consumption of lemon peel extract has some beneficial effects for childhood obesity; however, no considerable effect was documented on anthropometric measures and biochemical factors. Future studies with longer follow up are highly recommended.

Keywords: Overweight, Adolescents, Citrus Fuit, Trial.

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Introduction

Overweight and obesity are major health concerns, which are significantly increasing in children; their effects on health begin from early childhood (1). Childhood obesity is not limited to high-income countries, and it is rapidly increasing in developing countries in the Middle East as well (2).

The prevalence of overweight and obesity in Iranian children aged 6-18 years was reported 10.1% and 4.79% based on national cut-off points (3). The escalating trend of excess weight among Iranian young children is alarming (4).

Childhood obesity plays an important role as a predisposing factor for most non-communicable diseases including type 2 diabetes mellitus, hypertension, cardiovascular disease, as well as their predisposing factors as the metabolic syndrome and

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dyslipidemia (5,6). Obesity has also been shown to induce endothelial dysfunction and initiate atherosclerosis in childhood (7). One of the earliest detectable cellular responses in the formation of lesions of atherosclerosis is leukocyte adherence to the endothelium at particular anatomic sites in the artery wall. Members of the endothelial adhesion molecules, including intercellular adhesion molecule–1 (ICAM-1) and vascular cell adhesion molecule (VCAM-1), are considered as indicators of endothelial dysfunction (8).

An increasing body of evidence suggests that increase in oxidative factors accounts for a significant proportion of such complications (9). Implementation of treatment and preventive programs will be more effective when begun before adulthood (10).

Dietary intervention could play an important role in the control of obesity and its consequences (11). Epidemiological studies indicate an association between higher intake of dietary antioxidants and reduced risk of some chronic diseases (12,13).

In addition, endothelial dysfunction could be reversed by the use of superoxide scavenging agents such as vitamin C and flavonoids (14). Many studies have documented the effects of flavonoid-rich foods on the markers of cardiovascular disease risk (15-18).

Flavonoids, according to their structure, act as reducing agents and can serve as efficient chelators of transition metals that are involved in cellular oxidation reactions. The association of flavonoids, including citrus flavonoids with metabolic disorders and atherosclerosis has been linked to their antioxidant properties and to a reduction in oxidative stress (19-21).

An inverse relationship was found between flavonoid consumption and reducing cardiovascular risk factors, e.g., lowering blood pressure, weight control and improving dyslipidemia (15).

There is a paucity of information regarding the role of dietary intervention with fruits containing ample amounts of vitamin C and flavonoid on the control of cardiometabolic risk factors in the pediatric age group.

Citrus species fruits contain flavonoid, pectin and vitamin C. Moreover, naringenin is an antioxidant and anti-inflammatory agent, which is present in these kind of fruits (21). Various reports suggest that drinking generous amounts of a mixture of citrus juices improves the blood lipid profile, reduces oxidative stress, prevents atherogenic modifications of LDL cholesterol and platelet aggregation and improves HDL-cholesterol concentrations (17).

This study aimed to investigate the effects of the peels and external membranes of lemon (Citrus aurantifolia) on anthropometric measures and biochemical factors (blood sugar, lipid profile and markers of endothelial dysfunction) in adolescents with excess weight.

Methods

This triple-masked, randomized controlled trial was conducted in Isfahan, Iran. For ethical considerations, written informed consent, which was approved by the Vice-chancellery for Research and Technology of Isfahan University of Medical Sciences, was obtained from the parents or legal guardians.

Overall, 60 overweight or obese children and adolescents were studied for four weeks.

According to the revised growth charts of the Centers for Disease Control and Prevention (CDC), the body mass index (BMI) of 85th to 94th BMI percentile was considered as overweight, and that of ≥95th percentile as obese (22).

Participants aged 10-18 years who had BMI of more than 85th percentile were included in this study. Those with chronic diseases who took medication and those with history of smoking (active/passive) were not included in the trial.

Eligible participants were randomly selected from the clinics of the Child Growth and Development Research Center affiliated to the Research Institute for Primordial Prevention of Non Communicable Disease, Isfahan University of Medical Sciences,

Isfahan, Iran.

Eligible participants were randomly assigned into two groups of equal number receiving daily oral capsules containing lemon powder or placebo. Randomization was performed according to a random numbers table. The table was based on the recorded numbers of participants. Participants, practitioner and the investigators were kept blind about the grouping.

The patients in the first group received capsules containing lemon peel powder for a 4-week period. Other patients in the second group received placebo for a similar period. The study medications were prepared in the form of a capsule in the Pharmacognosy Department of Isfahan University of Medical Sciences. Well-dried peels and external membranes of lemon (Citrus aurantifolia) were used to prepare the powder. They were bought from Isfahan and Sari markets (the cities located in the center and north of Iran, respectively) and identified in the Isfahan Pharmacognosy department. For the placebo group, cornstarch powder was prepared (23). The capsules were prepared with the same color, shape and size. Secret codes were defined for each group and then the medications were packed and delivered to the center without any label to ensure that the study pediatrician, nurses, patients were kept blind about the content of the capsules.

A trained team of physicians and nurses conducted the physical examination of all participants. They measured weight and height, as well as waist and neck circumferences under standard protocols using calibrated instruments. BMI was calculated as weight (kg) divided by height squared (m^2). Fasting venous blood was obtained and fasting blood glucose (FBG), lipid profile including low-density lipoprotein-cholesterol (LDL-C), high density lipoprotein-cholesterol (HDL-C), triglycerides (TG) and total cholesterol were determined. Cell adhesion molecules (ICAM-1 and VCAM-1) were measured as markers of endothelial function. Systolic and diastolic blood pressures (SBP and DBP) were also assessed.

The trial was approved and registered in the Iranian Registry of Clinical Trials, which is a Primary Registry in the World Health Organization Registry Network (IRCT-ID: 201311231434N11).

Statistical Analysis: Data were analyzed using paired t-test, independent t-test, Chi-square tests and analysis of variance (ANOVA). We reported all values as means ± SD. P_{time}, P_{group} and $P_{time * group}$ were calculated for all variables. Variables percent change was defined as Variable $_{after\ treatment}$- Variable $_{baseline}$/ Variable $_{baseline}$, which was compared between the two groups. The statistical significance level was defined as $p<0.05$. SPSS for Windows software (version 20.0 SPSS Inc., Chicago, IL) was used for statistical analysis.

Results

Of the total 60 enrolled patients, 30 and 29 patients in the lemon group and control group completed the study, respectively. The mean (SD) age was not significantly different between the two groups (13.7±7.0 years in the lemon group vs. 13.2±9.2 years in the control group, respectively, p=0.24). Comparison of age and sex showed no statistically significant difference between the two groups (p=0.45 for age and p=0.65 for sex). In addition, no significant differences were detected between BMI and waist circumference in the two groups in the beginning of the trial. The details are presented in Table 1.

Table 1. Descriptive Statistics of the Study Participants

Variables	Groups Mean±SD		p
	Lemon	Placebo	
Body mass index(Kg/m^2)	23.38±3.82	24.72±3.67	0.17
Waist circumference (cm)	76.62±9.07	76.90±9.12	0.90
Neck circumference (cm)	31.72±3.13	31.53±2.25	0.79

Table 2. Variables in the Lemon and Placebo Groups at Baseline and after the Trial

	Groups		$P_{overall}$ [a]	P_{time} [b]	P_{group} [c]	$P_{time \times group}$ [d]	$P_{time \times age}$ [e]	$P_{time \times sex}$ [f]
	Lemon group	Placebo group						
BMI								
Before	23.38±3.82	24.72±3.67	0.177	0.024	0.154	0.050	0.487	0.702
After	22.74±3.83	24.36±3.53	0.098					
$P_{before,after}$ [g]	<0.001	<0.001						
Waist								
Before	76.62±9.07	76.90±9.12	0.906	0.267	0.839	0.442	0.908	0.541
After	75.87±9.07	75.60±9.10	0.912					
$P_{before,after}$ [g]	0.116	0.012						
Neck								
Before	31.72±3.13	31.53±2.25	0.799	0.317	0.376	0.037	0.369	0.343
After	31.66±3.02	31.07±2.26	0.402					
$P_{before,after}$ [g]	0.581	0.001						
SBP								
Before	103.37±9.55	98.90±8.49	0.063	0.829	0.086	0.067	0.045	0.189
After	99.53±8.12	97.41±6.76	0.281					
$P_{before,after}$ [g]	0.001	0.081						
DBP								
Before	65.67±8.78	66.90±8.06	0.578	0.087	0.330	0.019	0.090	0.531
After	63.97±7.90	67.41±7.75	0.096					
$P_{before,after}$ [g]	0.053	0.264						
FBS								
Before	88.47±9.53	86.31±6.94	0.326	0.508	0.662	0.093	0.577	0.922
After	86.33±5.98	87.03±6.20	0.660					
$P_{before,after}$ [g]	0.200	0.496						
Cholesterol								
Before	178.57±25.26	180.14±38.04	0.852	0.527	0.525	0.551	0.182	0.021
After	171.17±25.01	176.66±33.72	0.480					
$P_{before,after}$ [g]	0.043	0.396						
TG								
Before	95.07±49.19	142.76±90.55	0.016	0.767	0.125	0.002	0.232	0.252
After	114.33±67.66	116.07±68.49	0.922					
$P_{before,after}$ [g]	0.053	0.013						
HDL								
Before	50.37±9.62	54.62±11.59	0.130	0.541	0.031	0.161	0.632	0.582
After	49.60±9.26	56.86±10.85	0.008					
$P_{before,after}$ [g]	0.586	0.085						
LDL								
Before	109.17±26.03	97.26±31.38	0.123	0.630	0.339	0.194	0.357	0.186
After	98.67±24.51	95.00±27.13	0.594					
$P_{before,after}$ [g]	0.014	0.412						
ICAM								
Before	6600.63±1557.74	6997.93±1487.78	0.321	0.486	0.290	0.463	0.499	0.922
After	6596.66±1562.72	6832.52±2459.56	0.682					
$P_{before,after}$ [g]	0.750	0.617						
VCAM								
Before	18261.80±4070.73	19497.79±3083.30	0.195	0.994	0.115	0.424	0.612	0.448
After	18344.69±3248.25	19237.80±2794.17	0.288					
$P_{before,after}$ [g]	0.648	0.568						

All values are mean±SD ; [a] P value present a comparison baseline and end point values between two groups (computed by T-test), [b] P value demonstrate the effect of time (computed by analysis of the covariance);[c] P value demonstrate the effect of grouping (computed by analysis of the covariance);[d] P value demonstrate the time × group interaction (computed by analysis of the covariance)[e] P value demonstrate the time × age interaction (computed by analysis of the covariance)[f] P value demonstrate the time × sex interaction (computed by analysis of the covariance);g P values present comparison baseline and end point values within each group (computed by paired sample t test)

Before administrating the medication, no significant differences were observed in the mean SBP, TG, LDL-C, HDL-C, ICAM and VCAM between the two groups.

All mentioned parameters were compared again between the two groups after they had completed the one- month treatment period. After the trial, none of the assessed parameters showed any significant difference between the two groups (Table 2).

No significant difference was detected in the percent changes of variables between the two groups (Table 3).

Table 3. Percent Change in Variables in the Lemon and Placebo Groups at Baseline and after the Trial

Variables	Groups		p
	Lemon grou (Mean±SD)	Placebo group (Mean±SD)	
BMI (%)	-2.80±2.97	-1.41±1.74	0.105
Waist (%)	-0.94±3.20	-1.65±3.25	0.845
Neck (%)	-0.14±1.89	-1.46±2.12	0.911
SBP (%)	-3.50±4.70	-1.28±4.28	0.223
DBP (%)	-2.19±6.97	0.90±3.98	0.038
FBS (%)	-1.72±9.32	1.09±6.49	0.398
Cholesterol (%)	-3.51±11.51	-1.01±11.12	0.908
TG (%)	25.10±53.30	-8.16±40.04	0.821
HDL (%)	-0.23±15.43	5.46±15.96	0.821
LDL (%)	-7.25±21.76	-0.89±15.96	0.228
ICAM (%)	5.10±28.93	-1.30±35.27	0.478
VCAM (%)	4.69±22.16	-0.07±22.16	0.478

All values are mean±SD

Discussion

This trial showed some beneficial effects of citrus fruit for childhood obesity. The results of within-group analysis demonstrated a slight reduction in BMI, LDL-C and SBP in the lemon group. However, no between group difference existed in the studied variables.

Citrus flavonoid and Vitamin C, which are a main component in citrus species, have marked potentiality in lowering lipid and lipoprotein and can slow the progression of atherosclerosis and endothelial dysfunction (22). Pectin as a soluble fiber of citrus fruits has also mild hypocholesterolemic effects (23).

We did not observe any considerable differences in within-group and between-group analysis for HDL-C, total cholesterol and the LDL-C. This finding is in line with the results of some other studies in which the effect of other types of diets were investigated (24). Within-group analysis of our study also confirmed the results of a previous study conducted on 26 obese children aged 7-13 years (25). One possible reason for a lack of change in the blood lipids in both lemon group and the placebo group is that the adolescents' blood lipids were in the normal range at baseline. These outcomes may have been different if dyslipidemia was more prominent in the study participants (26). Future studies should focus on the link between biochemistry and physiological pattern of the individual's body.

Because of molecular and cellular alterations in adipose tissue, obesity is closely related with various inflammatory processes. Several pro-inflammatory factors are produced in adipose tissue as the body fat increases. Additionally, clinical and experimental data have demonstrated a link between systemic inflammation and endothelial dysfunction. It is well documented that disturbed endothelial function may be an early marker of an ongoing atherosclerotic process (27).

As a result, serum markers of inflammation and oxidative stress are associated with the early inflammatory processes of atherosclerosis (28).

Vitamin C inhibits peroxidation of membrane phospholipids and acts as a scavenger of free radicals. Moreover, it is needed for the synthesis of several hormones and neurotransmitters. Supplementation of vitamin C may improve the function of the human immune system and controlling inflammation such as antimicrobial and natural killer cell activities, lymphocyte proliferation, chemotaxis and delayed-type hypersensitivity (29). In previous studies, it has been documented that antioxidant therapy through supplementation of vitamins can improve the endothelial function in the pediatric age group (30).

It was expected that lemon, which is rich in vitamin C and flavonoids, could affect endothelial markers such as ICAM and

VCAM. Nevertheless, there are some differences between the results of our study and those of some previous studies. Endothelial markers in our study showed no significant differences either in between groups or within group analysis. One of the main reasons for this indifference was related to the duration of the treatment and the needed time to observe the outcomes, as well as the young age of the participants.

Study Limitations and Strengths: The study population was relatively small, and this could be regarded as the main reason why significant between-group differences of assessed parameters are suggested to be studied in future investigations. Perusing this further, the treatment period in this trial was limited to one month. This period may not have been sufficient for detection of all significant or non-significant between group differences. However, this period has resulted to beneficial effects in some previous studies. Moreover, because of low compliance of participants, we could not extend the study period.

In addition to its novelty in the pediatric age group, this study had strengths in a number of ways: First, this study was a triple masked and placebo controlled trial, which would reduce the selection and observational biases. Secondly, this study was a randomized trial, which could lower the effects of potential existing confounding variables.

Conclusion

This study revealed that consumption of lemon peel extract has some beneficial effects for obese children and adolescents. However, no significant effect was documented on anthropometric measures, cardiometabolic risk factors and markers of endothelial function. Future studies with longer follow up are highly recommended. Furthermore, extensive lifestyle change should be emphasized for controlling childhood obesity.

Conflict of Interest

The authors declared there is no conflict of interest.

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