

Original Article

Effects of Radio Frequency and Ultrasound Cavitation Therapy on Serum C-reactive Protein and Pro-oxidant-Antioxidant Levels

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Abstract

Background: A combination of radio-frequency (RF) and ultrasound cavitation (UC) has been reported to reduce indices of obesity. In this study, we aimed to investigate the effect of a combination of these techniques on anthropometric indices, pro-oxidant-antioxidant balance (PAB), and serum high-sensitivity C-reactive protein (hs-CRP).

Materials and Methods: This randomized clinical trial was performed on 50 healthy women between January 2014 and June 2014 in Ghaem Hospital, Mashhad, Iran. Participants were randomized to one of two groups, both of which received a low-calorie diet containing 500-kcal energy deficit per day. The trial group included twenty-five subjects who were assigned to the combined treatment of RF and ultrasound cavitation program of abdomen and flank areas. There were twenty-five control subjects who received the low calorie diet alone. Biochemical markers, including serum hs-CRP and PAB values, and anthropometric indices were measured in the intervention group and healthy controls.

Results: For both the intervention and control groups, waist circumference was reduced significantly by 3.76 ± 1.69 and 2.40 ± 1.04 , respectively ($P < 0.05$). In addition, abdominal circumference was reduced by 9.5 ± 2.66 and 3.12 ± 1.88 , in these groups, respectively ($P < 0.001$). Decrement of PAB level in the intervention group, and its increment in the control group, were not significant ($P > 0.05$). In addition, reductions of hs-CRP and PAB between the two studied groups during five weeks of study were not significant ($P > 0.05$).

Conclusion: Although there were significant reductions in anthropometric indices following treatment with RF and UC, the effects on serum PAB or hs-CRP were no significantly different, compared to the control group. Further studies are needed to confirm the beneficial effect for the use of these techniques.

Keywords: Obesity indices, PAB, hs-CRP, radiofrequency, ultrasound cavitation

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Introduction

One of the major public health issues is obesity. In 2008, an estimated 1.46 billion adults worldwide were overweight.¹ According to a recent World Health Organization (WHO) report, 56.8% and 46% of women and men were overweight, respectively.²

An increased prevalence of major cardiovascular risk factors such as hypertension, hyperlipidemia, diabetes and cardiovascular diseases are associated with overweight and obesity.^{3,4}

Oxidative stress is an imbalance between the production of

pro-oxidants and antioxidant defenses in favor of pro-oxidants is usually associated with elevated level of reactive oxygen species (ROS) formation, and plays a critical role in the development and pathogenesis of cardiovascular diseases related to metabolic syndrome and obesity.⁵⁻⁷ Whilst, there are many available methods for measuring oxidative stress, but there is no agreement on the universally accepted method. Previous investigations of serum pro-oxidant-antioxidant balance (PAB),^{6,7} have indicated that this may be a simple and useful method for assessing this. Hence we used this method which can measure the balance of oxidants and antioxidants simultaneously, using 3,30,5,50-tetramethylbenzidine (TMB) as previously described,⁸ using a single chromogenic end-point. The PAB assay can be calibrated against known oxidants and antioxidants. A linear decrease and increase of PAB value has been observed, respectively in association with increasing major antioxidants (like bilirubin, albumin, vitamin C, Trolox, glutathione, uric acid and ceruloplasmin) and pro-oxidants (tertbutylhydroperoxide, hydrogen peroxide, chloramine T and hypochlorite) in vitro.^{8,9}

High concentrations of serum C-reactive protein (CRP) are associated with systemic inflammation.¹⁰ Previous studies have shown a potential role of high sensitive (hs)-CRP in cardiovascular disease, and high concentrations have been reported in Iranian obese populations.^{11,12}

RF and ultrasound cavitation methods are practical procedures

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for mobilizing stored fat with the same efficacy as liposuction. In addition, these methods are less invasive, and therefore have fewer complications compare to liposuction.^{5,13}

Other modes of treatment for managing obesity and overweight include: behavior therapy, dietary change, pharmacological treatments and surgical methods.¹⁴ There has recently been a trend for using non-invasive techniques such as external laser therapy, Radio frequency (RF), cryolipolysis, injection lipolysis and external ultrasonic energy devices.¹⁵

There is a lack of randomized clinical trials data on the efficacy and safety of Radio frequency and ultrasound technology. Furthermore, there is little data evaluating their effects on serum hs-CRP and PAB.

In this study, we aimed to investigate the combined effect of RF and ultrasound technology on anthropometric indices, pro-oxidant-antioxidant balance (PAB), and serum high-sensitivity C-reactive protein (hs-CRP).

Method and Material

Subjects

This randomized clinical study was carried out between January 2014 and June 2014 at Ghaem Hospital, Mashhad University of Medical Sciences, Mashhad, Iran, and was registered on the Iranian Registry of Clinical Trials (IRCT) with a registration number of IRCT 2014042817475N1.

Fifty healthy women were recruited through a public advertisement, and were provided with information about the study by both verbal explanation and written consent form. The Ethics Committee of Mashhad University of Medical Science was approved all procedures of this study. The methods, population and sampling have been presented in detail previously.¹⁶

Our inclusion criteria were obese women between 18 to 65 years old (body mass index (BMI) between 25 – 29.9 kg/m²). Exclusion criteria were: any systemic or local inflammation or infection of the area to be treated, pregnancy or lactation, diabetes, cardiovascular diseases, and any consumption of topical steroids or phototherapy sensitive medicines during the last six months. In addition, women with implantation of a pacemaker device, and any systemic diseases, as well as those who did not agreed to participate in the study, or wished to withdraw from the study at any time, were excluded.

A trained nurse collected the demographic, socioeconomic level and medical history by questionnaires. Furthermore, anthropometric indices, including body weight (BW), body mass index (BMI), and body fat mass (BFM) were measured using a Tanita BC-418 body composition analyzer (Tanita, Tokyo, Japan) according to the standard protocol.¹⁷ Body weight and height were measured with the participants wearing light indoor clothing without shoes. Waist circumference (WC) was measured at a level midway between the costal margin and the iliac crest at the end of a normal expiration.

Intervention Procedure

Participants were randomized into one of two groups (25 interventions and 25 controls) by randomization computer-generated randomization method.

Both groups were advised about a low-calorie diet containing 500-kcal energy deficit per day, below the individual's daily energy requirements for five weeks.

Radiofrequency and focus ultrasound devices were utilized by trained technicians, only in the intervention group. Devices were operated twice weekly by the same technician with each device used once a week. Each intervention session took forty minutes. Any adverse effects, including erythema, pain during treatment or blistering were recorded.

Blood sampling and fasting blood glucose analysis

Blood samples were taken from each patient for analysis after 12 hours of fasting. Blood samples were centrifuged at 5,000 g for 15 minutes, at 4 °C. After separation, aliquots of serum were frozen at -80°C until analysis. Fasting blood glucose (FBG) concentrations was measured enzymatically with the use of commercial kits using the BT-3000 auto-analyzer machine (Biotechnica, Rome, Italy). hs-CRP was measured by a polyethylene glycol (PEG)-enhanced immunoturbidimetry method using an Alcyon analyzer (Abbott, Chicago, IL, USA).

Pro-oxidant-antioxidant balance (PAB) assay

The following reagents were prepared in double distilled water for Pro-oxidant-antioxidant balance assay: TMB powder (3,3',5,5'-tetramethylbenzidine, Fluka), peroxidase enzyme (Applichem: 230 U/mg, A3791, 0005, Darmstadt, Germany), chloramine T trihydrate (Applichem: A4331, Darmstadt, Germany), hydrogen peroxide (30%) (Merck). All the other reagents used were reagent grade and were prepared in double-distilled water.¹⁸

Serum samples were used for measuring PAB values. A modified PAB was applied based on the previously described method.¹⁹ The standard solutions were first prepared by mixing different proportions (0% – 100%) of 250 µM hydrogen peroxide with 3 mM of uric acid (in 10 mM NaOH). Sixty mg of TMB powder was dissolved in 10 mL of DMSO. The TMB cation was prepared by adding, 400 µL of TMB/DMSO to 20 mL of acetate buffer [0.05 M buffer, pH 4.5]. Fresh chloramines T (70 µL, 100 mM) solution was then added to this, mixed well, and then incubated for 2 hours at room temperature in a dark place. Twenty-five U of peroxidase enzyme solution was added to 20 mL of the TMB cation, dispensed in 1 mL aliquots and stored at -20°C. In order to prepare the TMB solution, 200 µL of TMB/DMSO was added to 10 mL of acetate buffer [0.05 M buffer, pH 5.8]; the working solution was prepared by mixing 1 mL TMB cation with 10 mL of TMB solution, incubated for 2 minutes at room temperature in a dark place and immediately used. Ten µL of each sample, standard or blank (distilled water) was mixed with 200 µL of working solution, in each well of a 96 well plate, which was then incubated in a dark place at 37°C for 12 minutes. At the end of the incubation time, 100 µL of 2M HCl was added into each well and measured in an ELISA reader at 450 nm with a reference wavelength of 620 or 570 nm. A standard curve was provided from the values relative to the standard samples. Values of the PAB are expressed in arbitrary HK units, which represent the percentage of hydrogen peroxide in the standard solution.

Statistical Analysis

SPSS for Windows, version 16 (SPSS Inc., Chicago, IL, USA) was used in all statistical procedures. Data were expressed as means ± SDs. Normality of data was analyzed by the Kolmogorov-Smirnov test. For quantitative data, the independent *t*-test and the paired *t*-test were used. Furthermore, Wilcoxon and Mann-Whitney tests were used when the data were not nor-

normally distributed. All data were presented as mean \pm SD in each group. P value < 0.05 was considered as statistically significant.

Results

As shown in Table 1, there was no significant difference in age, anthropometric indices, and FBG between the intervention and control groups at baseline ($P > 0.05$). Furthermore, there were no significant differences between the intervention and control groups at baseline, or at the end of the study in FBG; these values were 81.80 ± 7.54 and 79.64 ± 6.39 in intervention and control groups, respectively. The changes of FBG for each group and between the groups did not show significant differences.

Table 2 shows the changes in anthropometric indices of BMI, WC and AC in the intervention and control groups. There was a significant reduction in these measures of adiposity during the study period in both groups. In addition, there were 9% and 5% reduction in size of the AC after five weeks in the intervention and control groups, respectively ($P < 0.001$). The mean reductions of WC were 3.76 ± 1.69 cm and 2.40 ± 1.04 cm in the intervention and control groups, respectively ($P < 0.001$). No adverse effects, such as erythema, pain during treatment or blistering were detected.

PAB and hs-CRP assays

PAB levels of the two study groups in the intervention and control groups before the study periods were 150.15 ± 34.96 (HK unit) and 147.56 ± 36.58 (HK unit), respectively. There was no statistical difference regarding PAB levels before the study with similar serum PAB values ($P = 0.80$). Despite a decrease in the PAB level in the intervention group [mean 148.47 ± 39.13 (HK unit)] and an increase in the control group [mean 161.74 ± 34.53 (HK unit)], there was no significant difference between the values of the intervention and control groups after the study ($P = 0.21$). As shown in Figure 1, changes of the PAB values after week 5 was not significant compared to the base values in either of the intervention and control groups ($P > 0.05$) (Figure 1).

Similarly, as it shows in figure 2, there was no significant difference at baseline values of hs-CRP between the two groups of intervention 3.09 ± 1.94 (mg/dL) and control 1.82 ± 1.75 (mg/dL) and at week 5 between these two groups, 0.92 ± 0.27 (mg/dL) and 1.28 ± 1.22 (mg/dL), respectively ($P > 0.05$). Furthermore, although hs-CRP fell significantly after 5 weeks in both groups, these reductions were not significant between the two groups ($P > 0.05$).

Table 1. Baseline demographic characteristics and anthropometric indices in studied participants

Variables	Group (Mean \pm SD)		P-value
	Intervention	Control	
Age, years	36.52 ± 8.56	35.32 ± 8.70	0.62
BMI kg/m ²	27.07 ± 1.55	27.61 ± 1.44	0.21
WC (Cm)	80.96 ± 5.03	81.80 ± 4.21	0.58
AC (Cm)	89.92 ± 6.99	92.90 ± 5.36	0.08
FBG	81.76 ± 7.71	82.48 ± 9.45	0.64

BMI = Body Mass Index, WC = Waist Circumference, AC = Abdominal Circumference, FBS = Fasting Blood Sugar

Table 2. Changes in BMI, WC and AC during the study

Variables	Group (Mean \pm SD)		P for changes (mean reduction)
	Intervention	Control	
BMI kg/m²			0.301
Week 0	27.61 ± 1.44	27.07 ± 1.55	
Week 3	27.01 ± 1.40	26.52 ± 1.68	
Week 5	26.64 ± 1.49	26.31 ± 1.65	
P-Value	$P < 0.001$	$P < 0.001$	
WC (cm)			0.896
Week 0	81.80 ± 4.21	80.96 ± 5.03	
Week 3	80.40 ± 4.10	80.20 ± 5.46	
Week 5	78.04 ± 4.31	78.56 ± 4.82	
P-Value	$P < 0.001$	$P < 0.001$	
AC (cm)			0.934
Week 0	92.20 ± 5.36	89.92 ± 6.99	
Week 3	88.79 ± 5.12	87.92 ± 6.85	
Week 5	83.40 ± 4.94	86.80 ± 4.42	
P-Value	$P < 0.001$	$P < 0.001$	

BMI: Body mass index; EC: waist circumference; AC: abdominal circumference

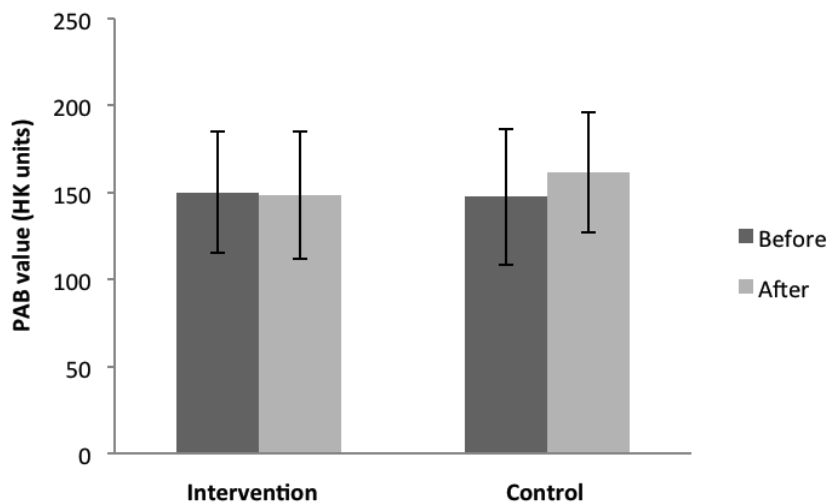


Figure 1. Pro-oxidant-antioxidant balance (PAB) values in intervention and control groups; no significant differences were observed before and after study between studied groups ($P > 0.05$).

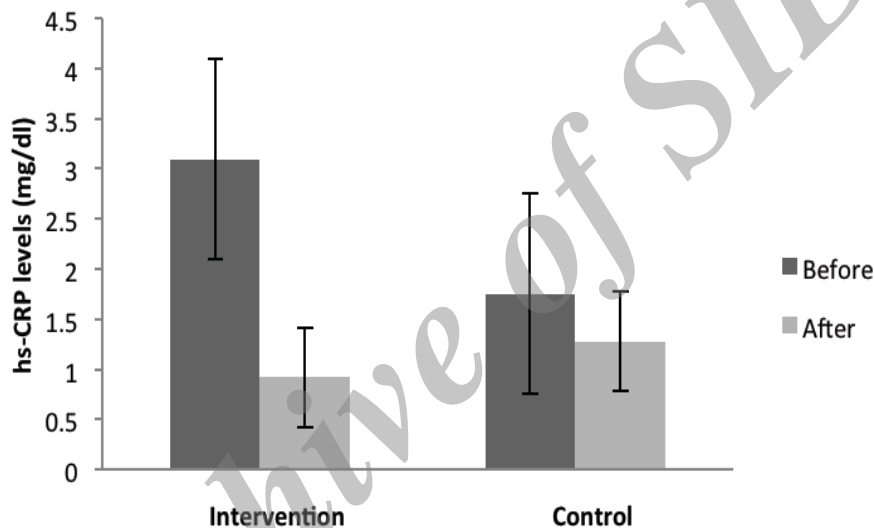


Figure 2. High-sensitivity C-reactive protein (hs-CRP) levels in intervention and control groups; no significant differences were observed before and after study between studied groups ($P > 0.05$).

Discussion

Among the anthropometric indices, we found BMI level in the intervention group had experienced a 22% decrease in BMI level compared to control group. However, we did not find any significant differences between the control and intervention groups. Similar to our study, a previous study by Milanese, et al.²⁰ found that BMI of 28 women who were treated by RF and ultrasound therapy for 10 weeks were decreased but this was not significant compared to the control group.

With respect to the effect of RF on abdominal circumference however, we did observe a greater reduction in the intervention group (9%) compare to the control participants (5%) and this difference was significant. However, previous studies had contrast^{21,22} or similar results^{23,24} to our study, but all last evaluations by different methods, including focused ultrasound, non-thermal focused and RF technology were observed decreasing level of AC

and WC in studied participants.

Analysis of fast blood glucose (FBG) in the intervention and control individuals during five-week study was not different from each other. In addition, Shalom, et al. in 2013 found similar results in obese women who were treated by focused ultrasound technology.²⁵

Serum hs-CRP concentrations reflect the inflammatory status. We investigated the hs-CRP level in obese participants, which revealed no significant decrease in the two groups. In contrast, a previous study²⁵ it was reported that there was a significant fall in serum hs-CRP after focused ultrasound treatment.²⁵ The reduction in serum hs-CRP were similar in both groups.

PAB is an oxidative marker that represents body oxidative stress. Razavi, et al.⁶ evaluated the cardiovascular risk factors and found that increased levels of PAB were observed in obese patients compare to non-obese patients. Coordination between PAB level and BMI besides the decrements of PAB level due to weight loss

suggests that obesity affects the inflammatory status and levels of oxidative stress. On the other hand, weight loss decreased inflammatory responses and oxidative stress. In our study, PAB variations during five weeks in intervention and control individuals were not significant. Our results show that despite the reduction of fat cells and release of their content in extracellular membrane, antioxidant to pro-oxidant balance was not significantly different. In addition, the average PAB and hs-CRP during the 5-week study were not significantly different between the two groups.

Limitations of this study include its relatively small sample size and the absence of a group treated with RF or ultrasound alone, not receiving a weight reducing diet.

Considering the advantages of applying RF and ultrasound technologies in addition to a low diet calorie regimen on a significant reduction in the abdomen and waist circumferences, changes of the PAB and hs-CRP values were not significant between the two groups, revealing the overall advantages of performing the newly introduced techniques in addition to the conventional regimen.

Authors Contribution

Mahsa Mohammadzadeh and Samira Nasrfard contributed equally to this work.

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