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## Effect of Body Acupuncture on Anthropometric Parameters, Lipid Profile, Inflammatory Markers and Adipokines among Obese Adults.

Laila Ahmed Abou Ismail<sup>1</sup>, Nagwa Abd El-Ghaffar Mohamed<sup>1</sup>, Solaf\* Ahmed Kamel<sup>1</sup>, Ghada Ali Helm<sup>2</sup>, Lylah M Labib<sup>2</sup>, Azza Sarry EL-Din<sup>2</sup> and Iman Ibrahim Salama<sup>3</sup>.

<sup>1</sup>Clinical and Chemical Pathology Department, <sup>2</sup>Complementary Medicine Department, <sup>3</sup> Community Medicine Department, National Research Center, Cairo, Egypt.

### ABSTRACT

Obesity is becoming increasingly common among general population and there are several modalities for the treatment of obesity one of which is acupuncture. Aim of this study was to examine the effectiveness of body acupuncture on anthropometric measurements, lipid profile, serum insulin, inflammatory markers and serum adipokines. A randomized controlled clinical trial was performed on 80 Egyptian obese patients. Subjects received acupuncture for 3-6 months in combination with a low-calorie diet. Subjects were assessed at the beginning and at the end of the study. There were significant higher levels of serum Hs-CRP, TNF- $\alpha$ , insulin, visfatin and resistin among obese subjects compared with controls ( $P < 0.05$ ). After acupuncture, there was highly significant decrease in mean weight, BMI, Fat percent, fat mass visceral fat rating, BMR ( $P < 0.001$ ), the mean level of serum Hs-CRP, TNF- $\alpha$ , insulin, visfatin and resistin, total cholesterol and triglyceride while, serum adiponectin was significantly increased ( $P < 0.001$ ). Serum adiponectin showed highly significant increase after acupuncture among mild and moderate obesity ( $P < 0.05$ ). Acupuncture has an effectiveness role on anthropometric measurements, lipid profile, serum insulin, inflammatory markers and serum adipokines among obese individuals.

**Keywords:** Acupuncture, lipid profile, inflammatory markers, adipokines.

*\*Corresponding author*

## INTRODUCTION

Obesity is a global public health problem that presents the characteristics of a pandemic due to its rapidly increasing incidence (1). Notably obesity promotes hyperglycemia, hyperinsulinemia as well as glucose intolerance and insulin resistance (2) that are part of the metabolic syndrome (3). Together with a balanced diet and physical exercise, anti-obesity drugs and surgery processes can help patients to lose weight. However, they usually produce adverse effects and weight regain is very common if patients do not strictly follow nutritional recommendations and come back to their sedentary life style (4).

Acupuncture has been practiced for thousands of years in China; represent a suitable therapeutic approach for individuals with obesity, without producing negative side effects and weight regain (5). Acupuncture involves the insertion of very fine, sterile needles at specific body points or "energy pathways." The inserted needles act to stimulate the release of endorphins, the body's natural "feel good" hormones. This can create a calming, relaxing effect, which counteracts the need for excessive eating brought about by increased stress, frustration or anxiety(6).

At cellular level, obesity is also related with activation of immunocompetent cells, such as macrophages, which produces a low-grade chronic inflammation characterized by abnormal cytokine production, increased acute phase reactants and other mediators and activations of a network of inflammatory signaling pathway. The predominant type of adipose tissue is white adipose tissue which is responsible for the synthesis of adipokines that are responsible for body weight control: leptin, adiponectin and resistin (4). Among variety of adipokines, resistin and visfatin are proposed as important pro-inflammatory mediators, which also interfere with the central regulation of insulin sensitivity. Adiponectin has been noted as an important antiatherogenic and antidiabetic protein (7).

Tumor necrosis factor-alpha (TNF- $\alpha$ ) is a pleiotropic molecule that plays a central role in inflammation, immune system development, apoptosis, and lipid metabolism, with numerous effects in adipose tissue, including lipid metabolism and insulin signaling. Circulating TNF- $\alpha$  is increased in obesity and decreased with weight loss. High sensitivity C-reactive protein (Hs-CRP) is a very sensitive marker of inflammation and is positively correlated with abdominal fat and closely correlated with increased risk of cardiovascular events (8). The aim of the study was to investigate the effect of obesity treatment by body acupuncture in combination with low calorie diet on anthropometric measurements, lipid profile, serum insulin and adipokines (visfatin, adiponectin and resistin).

## SUBJECTS AND METHODS

This study is an interventional clinical study and it was carried out during the period from April 2012 to May 2013 on 80 obese individuals aged 20-60 years. Approval of the ethical committee at National Research Center and a written consent was obtained from each subject in the study. Pregnant women and patients with serious medical conditions were excluded

A control group of 23 normal weight individuals of comparable age and sex was participated for comparison of laboratory results before the interventional study between each group of obesity and normal weight individuals.

**Anthropometric measurements** were performed for all patients by Tanita body composition analyzer BC-418 (Tanita Corporation ,1-Chome, Maeno-Cho, Itabashi-Ku, Tokyo, Japan) to determine (height, weight, body mass index, basal metabolic rate (BMR), fat %, fat mass, fat free mass, total body water). Degree of obesity was defined according to BMI cut-off points. The obese subjects were divided into 3 groups: mild obesity (BMI: 30-35 kg/m<sup>2</sup>), moderate obesity (BMI: 35 -40 kg/m<sup>2</sup>) and severe obesity (BMI: >40 kg/m<sup>2</sup>) represented 30% of obese subjects(9).

### Acupuncture protocol:

- 1- Classical Chinese body acupuncture and auricular acupuncture were only used. Other different forms of acupuncture like Laser acupuncture or other kinds were not used.
- 2- Combined effects of acupuncture with other therapies were excluded.

Body points are: Hegu (LI 4), Quchi (LI 11), Tianshu (St 25), Zusanli (St 36), Sanyinjiao (Sp 6), Daheng (Sp 15), Neiguan (P 6), and Fenglong (St 40). Neiting (St 44) and Taichong (Liv 3), Liangmen (St 21), Guanyuan (R 4), zhonwang Ren (12) (10). Acupuncture sessions was done twice weekly, each session was 30 minutes.

Auricular acupuncture: ear points are: Shen Men, mouth, Stomach, Sanjiao, liver, spleen, endocrine and Hunger points were chosen. They are weekly applied to each ear alternatively (11).

Obese individuals were followed-up for about 3 -6 months with a low calorie diet.

### Laboratory analysis

About 5 ml of fasting (12-14 hours) venous blood samples were taken from each subject participating in the study and was left to clot and centrifuged at 3000 xg for 5 minutes for separation of serum that was stored at -20°C. Serum cholesterol, triglyceride was determined using colorimetric methods on Olympus AU 400 supplied from Olympus Life and Material Science (Europe GmbH, Wendenstraße, Hamburg, Germany). For determination of HDL, phosphotungstic acid and magnesium ions are used for precipitating all lipoproteins except HDL fraction that was present in the supernatant and measured by auto analyzer. LDL was measured by Friedwald formula (12).

Quantitative determination of serum Hs-CRP in serum is done by a solid phase immunosorbent assay (13) and the kit was supplied from Monobind Inc. (100 North Pointe drive, Lake Forest, USA). Serum TNF- $\alpha$  was determined by ELISA kit supplied by Anogen (2355 Derry Road East, Unit 23, Mississauga, Ontario, Canada) (14).

Fasting serum insulin was determined using radio immuno assay (15). Serum visfatin is determined by competitive enzyme immunoassay (16) supplied from Phoenix Pharmaceuticals, Inc. (330 Beach Road, Burlingame, California, USA). Serum adiponectin is determined by ELISA kit supplied from AviBion (Ani Biotech, tiilitie, Finland) (17). Serum resistin is determined by sandwich enzyme immunoassay supplied from BioVendor (Im Neuenheimer Feld, Heideberg, Germany) (18).

### Statistical analysis:

Data was statistically described in terms of mean  $\pm$  standard deviation, frequencies (number of cases) and percentages when appropriate. Comparison of numerical variables between 2 study groups was done using Mann Whitney U test and Kruskal Wallis test with Mann Whitney U test as post-hoc when comparing more than 2-groups. For comparing pre and post acuapunctures, Wilcoxon signed-rank test was used for continuous variables and McNemar test for two related dichotomous variables for detecting changes in responses due to intervention. P value less than 0.05 was considered statistically significant. All statistical calculations were done using computer programs SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 15 for Microsoft Windows

## RESULTS

This study was performed on 80 obese subjects (74 females and 6 males), with age range 20-60 years with mean age  $43 \pm 12.2$  years. Regarding BMI, 22 subjects had mild obesity (27.5%), 34 had moderate obesity (42.5%) and 24 had severe obesity (30%). Steady pattern of weight gain was reported by about 40% of the studied population.

Table 1 showed serum inflammatory markers, serum insulin and serum adipokines before acuapuncture treatment. There were significant higher levels of serum Hs-CRP, TNF- $\alpha$ , insulin, visfatin and resistin among obese subjects compared with controls ( $P < 0.05$ ). There was no significant difference between the 3 groups of obesity in all the studied serum markers ( $P > 0.05$ ).

**Table 1: Comparison between different obesity groups and controls before-acupuncture as regards inflammatory markers, serum insulin and inflammatory adipokines**

Groups Parameters	Mild obesity N=22 X±SD	Moderate obesity N=34 X±SD	Severe obesity N=24 X±SD	Controls N=23 X±SD	P
Hs-CRP (µg/ml)	20.5± 10.9	18.9 ± 11.9	22.5 ± 11.9	5.8 ± 3.9	0.024
TNF-α (pg/ml)	16.7 ± 4.7	16.6 ± 4.3	17.5 ± 5.9	11.2 ± 2.2	0.045
Insulin (µIU/ml)	7.1 ± 4.4	7.7 ± 7.0	7.4 ± 4.2	3.9 ± 2.5	0.047
Visfatin (ng/ml)	57.5 ± 29.4	58.4 ± 25.2	50.4 ± 33.4	8.4 ± 6.3	0.004
Adiponectin (ng/ml)	10.1 ± 4.9	10.3 ± 4.0	10.2 ± 6.2	11.9 ± 3.7	0.169
Resistin (ng/ml)	30.6 ± 9.6	31.6± 11.8	32.6± 12.0	5.7± 2.8	0.000

P<0.05: significant.

P<0.01: Highly significant.

Table 2 showed the changes before & after acupuncture in different anthropometric measurement in obese subjects. There was highly significant decrease in mean weight, BMI, Fat percent, fat mass visceral fat rating and BMR post acupuncture P<0.0001.

**Table 2: Anthropometric measurement before and after acupuncture among obese subjects**

Parameters	Acupuncture		P
	Before	After	
Weight (Kg)	96.9 ±15.1	91.6± 14.6	< 0.001
BMI(Kg/m <sup>2</sup> )	38.6± 5.3	36.6± 5.0	< 0.001
Fat percent (%)	44.6± 5.2	42.2± 5.4	< 0.001
Fat mass	44.1± 11.2	39.6± 10.5	< 0.001
Visceral fat rating	11.8± 3.7	10.5± 3.7	< 0.001
BMR	6992.1± 926.7	6829.2 ± 836.4	< 0.001

BMR: basal metabolic rate

Table 3 demonstrated the changes in different laboratory parameters before and after acupuncture. There was highly significant decreased in the mean level of serum Hs-CRP, TNF-α, insulin, visfatin and resistin, total cholesterol and triglyceride post acupuncture P < 0.001. While, serum adiponectin was significantly increased post acupuncture, P < 0.001. There was no significant change in the level of serum HDL and LDL, P > 0.05.

**Table 3: Serum levels of inflammatory markers, serum insulin and inflammatory adipokines before and after acupuncture among obese subjects (Mean ± SD)**

Groups Parameters	Acupuncture		P
	Before Mean ± SD	After Mean ± SD	
Hs-CRP (µg/ml)	21.3 ± 9.9	14.9 ± 7.7	0.000
TNF-α (pg/ml)	16.9 ± 4.7	12.4 ± 3.1	0.000
Insulin (µIU/ml)	7.4 ± 6.1	5.8 ± 4.8	0.000
Visfatin (ng/ml)	55.3 ± 25.2	29.1 ± 19.0	0.000
Adiponectin( ng/ml)	11.1 ± 4.9	14.8 ± 5.8	0.000
Resistin( ng/ml)	30.8 ± 10.8	17.9 ± 6.5	0.000
Cholesterol(mg/dl)	186.8 ± 80.5	166.1 ± 70.4	0.000
Triglyceride (mg/dl)	133.3 ± 80.3	103.9 ± 62.4	0.000
HDL(mg/dl)	33.8 ± 20.7	37.7 ± 21.4	0.058
LDL(mg/dl)	99.7 ± 64.4	87.3 ± 56.9	0.165

Table 4 showed mean level of inflammatory markers, serum insulin and inflammatory adipokines before and after acupuncture as regards different groups of obesity. Among all groups, post acupuncture mean serum triglyceride, Hs-CRP, TNF-α, insulin, visfatin and resistin showed significant decreased compared

to pre-acupuncture  $P < 0.05$ . Mean serum cholesterol was significantly decreased post acupuncture among moderate and severe obesity,  $P < 0.05$  and serum adiponectin showed highly significant increase after acupuncture among mild and moderate obesity,  $P < 0.05$ . There was no significant difference between the 3 groups of obesity in all the studied serum markers either before or after acupuncture ( $P > 0.05$ ).

**Table (4): comparison of different laboratory parameters before and after acupuncture among obese individuals**

Groups Parameters	Mild obesity X±SD	Moderate obesity X±SD	Severe obesity X±SD	P
Cholesterol (mg/dl):				
Before	210.7 ± 50.4	190.8 ± 71.3	176.2 ± 96.6	0.885
After	195.0± 33.8	165.7±69.6*	131.8±89.6*	0.208
Triglyceride(mg/dl):				
Before	140.5 ± 51.5	125.4 ± 74.6	122.2 ± 87.0	0.572
After	117.9±43.8*	100.2±62.7**	90.9±78.5*	0.456
HDL (mg/dl):				
Before	45.3±13.5	44.9±11.5	40.1±14.4	0.654
After	72.5±57.1	54.4±33.1	31.4±25.2	0.010
LDL (mg/dl):				
Before	116.7±58.1	118.8±42.7	137.4±34.4	0.590
After	110.1±35.0	88.6±59.6	62.9±63.7	0.310
Hs-CRP (µg/ml):				
Before	20.5±10.9	18.9±11.9	22.5±11.9	0.024
after	15.4±7.8*	14.3±8.3**	18.2±7.4**	0.252
TNF-α (pg/ml):				
Before	16.7±4.7	16.6±4.3	17.5±5.9	0.045
After	12.5±2.2**	12.6±3.0**	13.2±3.8**	0.934
Insulin (µIU/ml):				
Before	7.1±4.4	7.7±7.0	7.4±4.2	0.541
After	4.7±3.3*	6.1±5.7**	6.5±3.8**	0.400
Visfatin (ng/ml):				
Before	57.5 ± 29.4	58.4 ± 25.2	50.4 ± 33.4	0.245
After	30.7± 25.1**	29.8± 17.7**	26.7±18.3**	0.892
Adiponectin (ng/ml):				
Before	10.1±4.9	10.3±4.0	10.2±6.2	0.512
After	14.9±5.6*	14.8±6.1**	13.3±4.1	0.507
Resistin (ng/ml):				
Before	30.6±9.6	31.6±11.8	32.6±12.0	0.256
After	18.9±6.7**	16.8±7.4**	19.1±4.9**	0.246

\*significant difference between pre and post acupuncture in each group of obesity  $P < 0.05$  \*\*highly significant difference between pre and post acupuncture in each group of obesity  $P < 0.01$

### DISCUSSION

The present study showed that the mean weight, BMI, fat percent, fat mass, visceral fat rating and BMR were highly significantly decreased after the acupuncture ( $P < 0.001$ ). This coincides with Abdi et al [19] and Hassan [9] who found greater efficacy of body acupuncture therapy on reduction of anthropometric parameters and decrease in visceral fat. Hsu et al [20] performed a randomized crossover trial in obese women and found that anthropometric parameters changed significantly by electro acupuncture when compared with sit-up exercise. The same results have been found by Lee et al [21]. However, another study reported no significant effect of auricular acupuncture in the treatment of obesity [22]. The reduction of BMI observed in the current study was in accordance with Tong et al [23], and Yeo et al [24]. They conclude that acupuncture therapy is one of the safe and effective methods for treatment of simple obesity.

Whereas this effect was not seen by exercise and diet alone. In addition, it has been shown that application of electro acupuncture at Zusanli (ST-36) and Neiting (ST-44) of the rat caused the increase in the electrical activity of ventral-medial hypothalamus in the obese rat, leading to activation of the satiety center [25]. Waist circumference is related to the subcutaneous fat tissue of the abdomen, and higher effects of body

acupuncture in lipolitic activity and enhancing lipid metabolisms could be attributed to the direct effects of body acupuncture in redistribution, lyses of fat tissue and reducing waist circumferences[20].

There was a significant difference between the three groups of obesity and the controls regarding serum Hs-CRP, serum TNF- $\alpha$ , serum insulin, serum visfatin and serum resistin, except for serum adiponectin that showed no significant difference before acupuncture treatment.

Concerning the effect of acupuncture on lipid profile before and after treatment, our results revealed positive changes (increase in levels of HDL-C and decrease in other parameters including triglycerides, total-cholesterol and LDL-C) were observed, however, the changes were significant in serum cholesterol & serum triglycerides and non significant in HDL&LDL after-acupuncture, indicating that whilst diet had important effects on the lipid profile but the combination of diet restriction and acupuncture therapy leads to a further improvement in lipid profile levels. These results agreed with Li and Wang [26], as they reported significant changes in total and LDL cholesterol during acupuncture therapy when compared with control subjects.

Our results agreed with other studies, a similar pattern of changes in triglyceride, total-cholesterol, LDL, and HDL changed have been reported following acupuncture [27,28]. Two of studies did not find any changes for HDL [27,29], this may be explained by application of different acupoints. It has been suggested that these changes in lipid metabolism may be caused by increase in the serum beta-endorphin levels [29]. Our results also agreed with study performed by Hassan et al [9], as they reported significant decrease in lipid profile after acupuncture treatment and they conclude that impaired lipid profile can be improved together with significant decrease in visceral fat by using acupuncture.

C-reactive protein (CRP) is a feature of systemic inflammation [30] and is positively associated with measures of adiposity such as BMI and waist circumferences as demonstrated by 2 large cross-sectional studies [31,32]. While obesity is related to higher levels of CRP [33]. Following weight loss reduction in CRP levels has been also reported [34]. It has been also suggested that adipose tissue releases the proinflammatory cytokines [35]. So it is logical that weight loss is associated with decrease in CRP levels. Although there are no studies assessing the role of acupuncture on Hs-CRP changes during weight loss, but there are some studies which have evaluated the changes in Hs-CRP levels in rheumatologic diseases. Some studies reported the inability of acupuncture to reduce the Hs-CRP [36,37] and some other studies not[28].

Our study showed significant reduction in serum Hs-CRP level after weight reduction by acupuncture treatment. Other studies by Hamid et al [38] found that changes in Hs-CRP levels were not different between cases and control, implying this notion that Hs-CRP changed independent of the effects of acupuncture.

In our study, acupuncture treatment could provide a reduction in serum TNF- $\alpha$  level in obese cases. Likewise, Tian et al [39] also found that serum TNF- $\alpha$  levels increased in rats with ulcerative colitis compared to normal rats. After that, they performed electro acupuncture on the St-36 point once a day for 10 days on these rats. As a result, they reported that there was a decrease in TNF- $\alpha$  level compared to the control rats. Yim and colleagues [40] reported a decrease in increased serum TNF- $\alpha$ , IL-6, and IFN- $\gamma$  levels as a result of 3 weekly sessions of acupuncture treatment. Using electric stimulation in these two studies with a long treatment period (5–9 weeks) or intensive (consecutive 10 sessions) treatment being applied to animals may have produced these results.

Adipokines are implicated in the pathogenesis of insulin resistance, metabolic syndrome and cardiovascular disease (CVD). They also play a role in the development of a local and systemic inflammatory state. Thus, circulating levels of adipokines (mainly adiponectin, leptin and resistin) can be used as biomarkers of obesity-related morbidity, particularly low-grade inflammation and insulin resistance (IR). Sustainable weight loss is the treatment of choice but is very difficult to achieve. However, even in absence of weight loss we can still break the obesity-inflammation vicious cycle that increases CVD risk. This can be achieved by normalizing adipokine levels. Adipokine levels might also be useful as markers of the efficacy of the treatment in obesity, IR and type 2 diabetes mellitus [41].

In our study after successful weight reduction there was marked reduction in serum cholesterol, serum triglyceride, serum insulin, serum visfatin and serum resistin and increase of serum adiponectin level and it was significantly different after acupuncture treatment. This finding is in accordance with study done by

Zhang [42] who declared that acupuncture can relieve insulin resistance, lower the lipid. The inhibitory effect of acupuncture on insulin resistance is somewhat related to a rise in lipid level and is thus an effective remedy for simple obesity.

Our study showed increase in serum adiponectin level after acupuncture treatment and this finding coincides with study done by Luo and Li [43] where they found that both electric and manual acupuncture can effectively lower blood Leptin content and raise blood adiponectin in simple obesity patients, which may contribute to its effect in reducing body weight.

Some studies concerning the association of visfatin with obesity and diabetes have brought up controversial results [44,45,46,47,48]. Few studies have been designed to explore changes on visfatin levels after weight loss. In morbidly obese patients, elevated visfatin concentrations were reduced after weight loss [44]. This is in accordance with our study that showed marked decrease in serum visfatin level nearly 40% of the starting level after weight reduction by acupuncture treatment.

Acupuncture is able to increase the levels of enkephalin, endomorphin-1, beta endorphin, serotonin, and dopamine and has immunomodulator effects on immune system and lipolytic effects on metabolism. These may be caused by the direct impression of body acupuncture on the adipose tissue of the abdomen, which means the greater losing of adipose tissue of the abdomen, leading to production of less inflammatory markers for the stimulation of the immune system. This role is suggested to be explained by the interaction between the autonomic nervous system and the immune functions and the brain as the communicator between 2 systems [49].

At the end, some potential reasons for these discrepancies among different studies could be due to the differences in age, race, obesity degree, and fat distribution. However, most studies have methodological limitations, including small sample size and inadequately controlled study design [27].

In conclusion, body acupuncture in combination with diet restriction was found to be effective for weight loss and also reduction of the obesity-associated risks factors, such as dyslipidemia. These effects can be achieved by other modalities but due to lack of adverse events and continued effects after the therapy, acupuncture could be used as a preferred or synergic treatment option for obesity control. Moreover, it was found that it has immunomodulatory effect on immune system by regulation of the levels of inflammatory markers and lipolytic effects on metabolism that means the greater losing of adipose tissue of the abdomen, leading to production of less inflammatory markers for the stimulation of the immune system.

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