Research Article

Betahistine and Metformin Reduce Weight and Affect Plasma Adiponectin and Nesfatin-1 Levels in Iraqi Obese Women

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Accepted on: 23-04-2016; **Finalized on:** 31-05-2016.

ABSTRACT

The aim of this study is to investigate the effect of metformin and betahistine along with lifestyle change on weight and plasma adiponectin and nesfatin-1 in obese women in Iraq. This study was carried out on 90 female patients, with an age range of 18-50 years, allocated into three groups. Patient's history was taken, and clinical examination was done to meet inclusion criteria. Serum transaminases and estimated glomerular filtration rate were tested at baseline to exclude hepatic or renal abnormalities. Each metformin and betahistine, along with lifestyle intervention highly significantly reduced weight, waist circumference, increased plasma adiponectin and decreased plasma nesfatin-1 after 12 weeks in obese women compared to pre-treatment values, and the changes elicited by metformin and betahistine (plus lifestyle change) were highly significant compared to placebo (lifestyle change alone). The results obtained in this study clearly demonstrated the beneficial effect of using metformin or betahistine to induce weight loss in obese women, and this weight reduction was negatively correlated with plasma adiponectin and positively correlated with plasma nesfatin-1, confirming the role of pharmacotherapy in targeting obesity and establishing the relation of weight loss with plasma adiponectin and nesfatin-1.

Keywords: Obesity, Lifestyle change, betahistine, metformin, adiponectin, nesfatin-1.

INTRODUCTION

In the last few decades, obesity became a global epidemic. The incidence of obesity is raising continuously and therefore, the associated morbidity, mortality and both medical and economical costs are expected to increase as well[1]. In Iraqi population, the prevalence of obesity reported by the WHO in 2005 was 8.3% and 19.1% for males and females respectively[2]. In 2008, another study concluded that obesity affects about 30% of adult population, with higher prevalence in women[3]. Obesity has become the primary cause of non-communicable diseases over the world[4], with high health and social costs[5]. The U.S. expenditure on obesity-related health care issues was $190 billion in 2005, twice than previous estimates[6]. Obesity increases cardiovascular risk, disturbs lipid profile, elevates plasma glucose and insulin and high blood pressure[7]. In particular, central obesity, which is characterized by the storage of the excess of fat in the upper part of the body, is strongly associated with the metabolic syndrome and cardiovascular disease[8]. Nesfatin-1 is an 82-amino-acid peptide initiated by post translational processing nucleobindin 2 (NUCB2), a 396-amino-acid protein remarkably conserved across mammalian species[9]. Another study found higher NUCB2/nesfatin-1 expression in adipose tissue in obesity[10]. Nesfatin-1 is widely distributed in the CNS and in peripheral tissues such as adipose tissue, pancreas, and stomach, and is involved in several physiological functions including food intake, appetite stimulation, and energy homeostasis[11]. Oh-I and co-worker confirmed that nesfatin-1 has a physiological role in regulating food intake, since nesfatin-1 injection into the third brain ventricle decreases significantly food consumption and consequently body weight, and the satiety induced by nesfatin-1 was independent of leptin, but, central administration of α -MSH increases NUCB2 mRNA expression in the hypothalamus[9]. Adiponectin is a protein hormone exclusively produced by adipose tissue and secreted into the bloodstream, and the levels of the hormone are negatively correlated with body fat percentage[12]. Adiponectin has a role in modulating a number of metabolic processes, such as glucose regulation and fatty acid oxidation[13]. Adiponectin, a major adipocyte-secreted adipokine, has an insulin-sensitizing and anti-inflammatory activity, and is down-regulated in obesity and its related pathologies[14]. Several strategies to increase adiponectin levels or adiponectin receptor activities are being investigated for the treatment of obesity-induced inflammation and insulin resistance[15].

Medications used in the study

Metformin

An anti-hyperglycemic medication used for the treatment of non-insulin-dependent diabetes mellitus, and is nowadays regarded the first line therapy in type 2 diabetes[16]. Its anti-hyperglycemic effect is by potentiation of insulin action via reducing insulin resistance, increasing peripheral glucose uptake and reducing gluconeogenesis weight[17,18]. Other effects of the use of metformin are lowering of systolic and diastolic blood pressure[19],

improving glucose and lipid metabolism, and reducing blood pressure in hypertensive, obese females[20].

Betahistine

A histamine receptor H1 (HRH_1) agonist and HRH_3 antagonist[21], that has been used to treat Meniere's disease since the early 1960s (22). Histamine H_3 are present in histaminergic (auto-receptors) and non-histaminergic (hetero-receptors) neurons, regulating release of histamine and other neurotransmitters like dopamine, norepinephrine or serotonin, betahistine increase the release of histamine and probably other neurotransmitters[23]. In post hoc analysis, betahistine had been shown to induce weight loss in non-Hispanic women[24]. Betahistine had been reported to reduce olanzapine-associated weight gain, and improved lipid profile in patients with schizophrenia, compared with control subjects[25].

PATIENTS AND METHODS

Ninety obese women, aged 18-50 years old, had been primarily enrolled in this study, and were divided into 3 groups. Medical history was taken from each patient.

A written consent from each patient to be involved in the study was obtained.

Inclusion Criteria

1. (Female) obese patients

2. Body Mass Index (BMI) equals or more than 30.

3. All are aged between 18-50 years, and pre-menopause.

Exclusion Criteria

1. Patients with renal insufficiency: Renal professionals consider the glomerular filtration rate (GFR) to be the best overall index of kidney function. GFR less than 60 represents CKD the National Kidney Foundation offers an easy to use on-line GFR calculator to estimate GFR[26,27].

2. Hepatic impairment: Aspartate aminotransferase (AST) or alanine aminotransferase (ALT) higher than two-folds the reference range[28].

3. Heart diseases (including unstable angina, myocardial infarction, transient ischemic attacks/stroke, clinically significant arrhythmia, congestive heart failure (increased risk of lactic acidosis), or cardiac valve abnormalities[29].

4. Patients with a history of seasonal allergy, asthma, or peptic ulcer[30].

5. Known allergy to one of the medications used in the study.

6. Pregnant or lactating women[29].

7. Known cases of type 1 or type 2 diabetes mellitus.

8. History of thyroid dysfunction, Cushing's syndrome or Addison's disease.

Treatment arms

Obese women were allocated into 3 groups, treated for 12 weeks as follows:

Group 1: metformin (1700 mg/d in 2 divided doses) + lifestyle intervention

Group 2: betahistine (96 mg/d in 3 divided doses) + lifestyle intervention

Group 3: control group: placebo + lifestyle intervention for 12 weeks

Lifestyle internevtion (Diet Program and Physical activity)

Diet Program

The patients were instructed to follow the diet regimen adapted by the obesity unit at Alkindy medical college (at which the study was done) which provides a low-calorie diet (LCD) of 1000-1200 kcal/day, the National Heart, Lung, and Blood Institute (NHLBI) Obesity Education Initiative recommended 1,200 to 1,600 kcal/day for men, and 1,000 to 1,200 kcal/day for women as a Low-calorie diet (LCD)[31].

Physical activity

The patients were instructed to practice a medium intensity exercise for 60-90 minutes/day. The Dietary Guidelines for Americans recommend that to lose weight, obese people have to participate at least 60 to 90 minutes of moderate- to vigorous-intensity daily physical activity, along with caloric intake restrictions[32].

Serum Adiponectin and Nesfatin-1 assay principles

Human Nesfatin-1 ELISA test

Antibody specific for Nesfatin-1 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any Nesfatin-1 present is bound by the immobilized antibody. After removing any unbound substances, a biotin-conjugated antibody specific for Nesfatin-1 is added to the wells. After washing, avidin conjugated Horseradish Peroxidase (HRP) is added to the wells. Following a wash to remove any unbound avidin-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of Nesfatin-1 bound in the initial step. The color development is stopped, the intensity of the color is measured. Use a serum separator tube, and permit samples to clot for 2 hours at room temperature or overnight at 4°C before centrifugation for 15 minutes. Remove serum and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles[33].

Human Adiponectin ELISA test

The Adiponectin in the samples binds to the first antibody coated on the microtiter plate. In the following step the second specific anti-Adiponectin-Antibody binds in turn to the immobilized adiponectin. The second antibody is

biotinylated and will be applied in a mixture with a Streptavidin-Peroxidase-Enzyme conjugate. In the following substrate reaction the turn of the color will be catalyzed quantitatively depending on the adiponectin level of the samples. Standards of the test kit are prepared from human adiponectin[34].

Statistics

The results represented as the mean ± standard deviation. Data were fed to the computer program. Statistical Package for Social Science SPSS version 16.0 under Windows Seven was used for analysis.

Normality test (Shapiro-Wilk test) was performed first, student *t*-test (paired) with p-value less than or equal 0.05 (P ≤0.05) were used to compare between pre- and post- treatment values within the same group, ANOVA test was used for comparing pre- treatment values among groups, and pre-intervention values were used as covariates for adjustment when comparing post-intervention results, using ANOVA test[35].

RESULTS

Baseline data (table 1) of the enrolled females who completed the study course showed no statistically significant difference among different treatment arms concerning participant's number, age, and body mass index. Liver transaminases and estimated glomerular filtration rate (e-GFR) results showed normal liver and kidney functions, with no statistically significant difference among obese women in this regard. Baseline serum levels of weight, waist circumference, plasma adiponectin and nesfatin-1 showed also no statistically significant difference among different treatment arms:

Table 1: Baseline characteristics comparison for enrolled patients: Results represented as mean ± Standard deviation (except for patient no.). NS means that there is no significant difference between the group's data.

Characteristic (Baseline)	Group 1 Metformin	Group 2 Betahistine	Group 3 Control	Significance
Participants No.	26	27	25	NS
Body Mass Index (kg/m^2)	38.2±3.30	37.7±4.0	37.08±3.28	NS
Age (years)	35.81±11.47	35.39±10.04	36.32±8.85	NS
Serum AST (IU/L)	22.69±7.2	21.3±8.0	22.32±5.8	NS
Serum ALT	22.88±7.3	21.41±6.15	22.52±5.2	NS
e-GFR (ml/min/1.73m^2)	84.69±12.9	84.70±11.3	84.80±8.3	NS
Weight (kg)	98.88 ± 12.46	97.77 ± 15.51	98.64 ± 11.4	NS
Waist (cm)	98.30 ± 7.59	97.81 ± 9.35	98.84 ± 9.37	NS
ADIPONECTIN (µg/mL)	13.36 ± 2.59	13.41 ± 3.37	13.51 ± 1.88	NS
NESFATIN-1 (ng/mL)	1.33 ± 0.10	1.31 ± 0.11	1.34 ± 0.087	NS

Table 2: Weight, waist and hormones changes. Data represent the change adjusted for baseline values. P-value of the paired t-test is used. P ≤ 0.001 represent a highly significant difference

Treatment	Weight (% of change)	P-value	Waist	P-value	Adiponectin	P-value	Nesfatin-1	P-value
Metformin	-9.26%	$1x10^{-13}$	-8.68	1×10^{-8}	+5.86	1×10^{-9}	0.19	1×10^{-9}
Betahistine	-9.65%	$6x10^{-14}$	-9.02	5×10^{-9}	+6.43	1×10^{-10}	-0.203	2×10^{-10}
Placebo	-1.93%	.017	-2.45	0.004	+1.18	0.003	-0.057	0.005

Table 3: Data represent the p-values of the pairwise comparisons. P > 0.05 represent no significant difference, P ≤ 0.001 represent a highly significant difference

Treatment	Weight	Waist	Adiponectin	Nesfatin-1
Metformin vs. Betahistine	0.272	0.480	0.092	0.728
Betahistine vs. Placebo	$3.2 x10^{-34}$	2×10^{-22}	3×10^{-25}	2×10^{-19}
Metformin vs. Placebo	$1.45 x10^{-32}$	5×10^{-21}	3×10^{-22}	2×10^{-19}

Results in table 2 showed that metformin (with lifestyle intervention) or (Group 1), highly significantly reduced weight and waist circumference, raised serum adiponectin and lowered nesfatin-1 after 12 weeks compared to pre-treatment values. After adjustment to baseline data, the percent of change of weight was -9.26%, waist change: -8.68cm, adiponectin change: +5.86 µg/mL, and nesfatin-1 change was -0.199 ng/mL. Results in table (2) showed also that betahistine (with lifestyle intervention) or (Group 2), highly significantly reduced

weight and waist circumference, raised serum adiponectin and lowered nesfatin-1 after 12 weeks compared to pre-treatment value. After adjustment to baseline data, the percent of change of weight was -9.65%, waist change: -9.02 cm, adiponectin change: +6.43 µg/mL, and nesfatin-1 change was – 0.203 ng/mL. Simultaneously; control group (lifestyle intervention only) or (Group 3), significantly reduced weight and waist circumference, raised serum adiponectin and lowered nesfatin-1 after 12 weeks compared to pre-treatment value; and after adjustment to baseline data, the percent of change of weight was -1.93%, waist change: -2.45 cm, adiponectin change: +1.18 µg/mL, and nesfatin-1 change was –0.057 ng/mL.

Pairwise comparison between each two groups demonstrated no statistically significant difference between metformin and betahistine group effects on all the measured parameters (p-values are more than 0.05), whereas a highly statistically significant difference between each metformin group and betahistine group effects were found when compared to control group (p-values are extremely less than 0.001), as shown in table (3).

DISCUSSION

Metformin and betahistine with lifestyle intervention caused a highly statistically significant reduction in weight, waist circumference and plasma nesfatin-1 with a highly significant elevation in plasma adiponectin. The control group (lifestyle intervention only) caused a statistically significant reduction in weight, waist circumference and plasma nesfatin-1 with a highly significant elevation in plasma adiponectin. Both metformin and betahistine treatment groups achieved a highly statistically significant difference compared to the control group, whereas no statistically significant difference between metformin and betahistine treatment regarding all of the above mentioned parameters. A review article stated that short-term lifestyle intervention program results in weight loss of 2 to <5%[36]. However, better improvement in risk factors observed with greater weight losses rates of 5 to 10%, which were associated with highly significant improvements in CVD risk factors[37].

The importance of measuring how much the waist circumference is reduced belongs to the impact of weight loss on affecting abdominal fat in particular. A waist circumference of more than 88 cm for women is predictive of early metabolic syndrome[38]. In 2009, a study found similar findings to the present study regarding weight reduction using metformin 1700 mg/day for 3 months[39]. Another study used metformin in an average of 2200 mg/d found a highly significant reduction in weight (p=0.0002) in obese patients (138 women, 16 men) with BMI of (35.4±6.5) over 6 months compared to control patients[40]. Metformin increases skeletal muscle AMP-Kinase activity, key enzyme regulating energy metabolism, increasing energy supplies of intramuscular triglycerides stores in physically active individuals, and exercise increases dependence on fat compared with rest, and the primary mechanism for this upregulation in lipid utilization is via skeletal muscle AMP-Kinase activity[41]. Metformin reduces appetite by decreasing neuropeptide Y (NPY), and agouti- related protein (AgRP), the orexigenic peptides in the hypothalamus. Centrally administered metformin prevents ghrelin-induced activation of hypothalamic adenosine mono-phosphate-activated kinase (AMPK), mediating anorectic effects[42].

Betahistine has a direct histamine H_1R agonistic activity[43], and upregulates histamine turnover and release, by blocking presynaptic histamine H_3 auto-receptors, and provoke histamine H_3 receptor downregulation[44]. Histamine can decrease body weight and obesity in diet-induced and genetically obese mouse by affecting both food intake and energy expenditure[45]. As a histamine HRH3 antagonist, it can increase norepinephrine's release, which might appear to be a useful therapeutic target for activating the brown adipose tissue (BAT), binding of norepinephrine to β3–adrenergic receptors within brown adipose tissue increases cAMP, which stimulates protein kinase A (PKA), leads to PGC-1α binding to the uncoupling protein-1promoter and stimulates its expression, dissipating energy as heat[46]. The results of the present study are consistent with a study by Barak N results, but inconsistent with another study which concluded that betahistine did not significantly affect food intakes[47]. The present study states that highly significant difference in weight reduction caused by metformin or betahistine compared to control might be reflected by a highly significant difference in increasing adiponectin level. This result is consistent with several clinical trials concerning obesity, that weight losses were significantly correlated with an increase in adiponectin level[48,49]. Adiponectin counteracts the obesity-associated abnormalities by suppressing gluconeogenesis, augmenting fatty acid oxidation, and inhibiting monocyte adhesion in blood vessels[50]. These actions of adiponectin are thought to be through the activation of AMPK and modulation of inflammatory signals[51]. A study revealed that treatment with 4 months of metformin therapy significantly lowers circulating adiponectin levels (p=0.026), and improved CV outcomes, and the lowering of adiponectin may partly explain the cardio-protective effects of metformin[52]. Up to knowledge, no previous studies that had evaluated the effect of betahistine on plasma adiponectin level. However, it had been documented that BMI correlates negatively with plasma adiponectin, and weight loss significantly increases circulating levels[53]. Since betahistine caused the highest weight reduction percent, this might possibly explain its highest reduction in plasma level of adiponectin. The present study shows that weight losses are directly proportional to decreased nesfatin-1 level, the highly significant difference in weight reduction caused by metformin or betahistine compared to control that may be reflected by a highly significant difference decrement in nesfatin-1 level. A positive correlation

between plasma Nesfatin-1 and BMI has been reported by a number of studies[10].

A recent study stated that the positive correlation of nesfatin-1 reported by many studies, along with the negative correlation of orexin-A with BMI recommend that these neuropeptides play a role in the protective mechanism in maintaining the nutritional status, and have a role in the regulation of food intake[54].

However, some authors have found a negative correlation between plasma nesfatin-1 levels and BMI[54]. Nesfatin-1 is a peptide produced by peripheral tissues, in addition to the central and peripheral nervous system, it suppresses food intake and is involved in the control of energy homeostasis related with water intake and food intake[9]. Nesfatin-1 suppresses food intake independently from the leptin pathway and enhances insulin secretion from pancreatic beta cells, drawing an attention as a potential new therapeutic agent for the management of obesity and diabetes mellitus[54]. In the present study, nesfatin-1 levels rapidly drop with caloric restriction and weight loss. Nesfatin-1 is a satiety molecule, and showed a positive correlation with BMI. This could be explained as an adaptive physiological response in order to decrease satiety, stimulate food intake, and decrease energy expenditure.

This behavior is similar to that of leptin, which rapidly drop with diet restriction and weight loss as an adaptive physiological compensatory reflex to starvation aimed at increasing appetite and decreasing energy expenditure[54].

Taken together; there may be multiple pathways that might explain how betahistine and metformin can reduce weight in obese women with central obesity and affect plasma level of adiponectin and nesfatin-1, the hormones that have an impact on food intake regulation.

Acknowledgement: Thanks to the members of the obesity research and therapy unit in Al-Kindy Medical College for their kind support and cooperation; in addition to the members of the department of pharmacology and therapeutics, College of Medicine, Al-Nahrain University for their encouragement and assistance.

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Source of Support: Nil, **Conflict of Interest:** None.