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DIETARY THERAPY OF OBESITY: EFFECT ON SOME HORMONAL AND BIOCHEMICAL BLOOD INDICES

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ABSTRACT

It is now clear that the presence of obesity substantially increases the risk of related co-morbidities such as insulin resistance, diabetes, dyslipidemia, hypertension and others. The objective of this study was to measure adiponectin, insulin hormones, and homocysteine concentrations in obese Egyptian women before and after diet therapy that consisted of a hypo-caloric regimen supplemented with a formula rich in dietary fiber, folate and betaine. This study investigated serum adiponectin, insulin, homocysteine, lipid profiles, haemoglobin and homeostatic model assessment (HOMA) value in twenty eight volunteer obese women, whose mean age was 47.86±2.18 years and body mass index (BMI) was 34.10± 0.95 kg/m^2. The studied period was 8 weeks divided into two phases, 1 and 2 of 4 weeks each. In the first phase, the women consumed a hypo-caloric diet (900-1000 Kcal/day) plus the supplement made from highly extracted wheat (82%) composited with ground peanuts at a 50:50 ratio, prepared as cookies of; 20 g each. Two cookies were consumed at breakfast and one at dinner, to replace the bread carbohydrate content. In the second phase, the same subjects consumed only the hypo-caloric diet. Results showed that fasting serum glucose, insulin, homocysteine concentrations and HOMA values were reduced significantly ($P<0.01$) at the end of the 1st phase, while adiponectin hormone was slightly decreased (1.3%). Homocysteine concentration increased significantly ($P<0.05$) at the end of the 2nd phase, while the other parameters showed only numerical increase. Adiponectin was positively correlated with high density lipoprotein cholesterol (HDL-C) at $P<0.01$ at all phases of the study, while insulin and HOMA were negatively associated at the start of the study. Homocysteine was positively correlated ($P<0.05$ and $P<0.01$) with cholesterol, low density lipoprotein cholesterol (LDL-C) and glucose at the basal test, than with systolic blood pressure (SBP), diastolic blood pressure (DBP), and HOMA ($P<0.01$) at the end of the 1st phase. In conclusion, the weight reducing diet supplemented with the dietary fiber rich formula in the short-term might have a beneficial effect on body weight, insulin and homocysteine. Adiponectin showed minor changes, but its role against dyslipidemia could be suggested.

Key words: Adiponectin, Homocysteine, Insulin resistance, Obesity, Peanuts

INTRODUCTION

Insulin resistance is defined as a subnormal response to both endogenous and exogenous insulin [1]. It is characterised by a decreased sensitivity of the target tissue to the action of insulin, by elevated blood glucose concentration, and increased hepatic production of atherogenic lipids [2]. Adiponectin has now been added on the list as a new player in the field of obesity-related insulin resistance and atherosclerosis. Adiponectin gene expression in white adipose tissue is decreased by obesity, glucocorticoids, β-adrenergic, cold exposure, adrenalectomy and insulin like growth factor 1(IGF-1) [3].

The main mechanism of action of adiponectin is directed to a protective role against atherogenic and insulin resistance processes [4]. Adiponectin is an adipokine that exerts a potent insulin-sensitizing effect by binding to its receptors (AdipoR) such as AdipoR1 and AdipoR2, leading to activation of AMP-activated protein kinase, PPAR-α and presumably some other unknown signalling pathway [5].

On the other hand, homocysteine is an important risk factor for cardiovascular disease. There is a growing amount of clinical evidence indicating that mild to moderate fasting hyperhomocysteinemia is an independent risk factor for atherosclerosis [6]. A study carried out on 149 overweight and obese volunteers showed a significantly higher level of serum homocysteine in the overweight subjects. They found that serum folic acid in the overweight and obese was significantly lower than in the control subjects [7].

Homocysteine is controlled both by mutations in its regulating enzymes and the B vitamins; folic acid, B_{12} and B_6 (pyridoxine). Furthermore, some evidence suggests an increase in plasma homocysteine concomitant to weight loss, apparently due to a decrease in the regulatory B vitamins [8]. In this context, nut is a rich source of vitamins, minerals, antioxidants and plant proteins which could be also beneficial [9]. It has been also reported that egg yolks are the richest source of choline, followed by soybeans, spinach, beet and that whole wheat products are primary sources of betaine; the metabolite of choline [10]. Choline and betaine work together in the cellular process of methylation, which is not only responsible for removal of homocysteine, but is involved in turning off the promoter regions of genes involved in inflammation [11].

The aim of this work was to highlight the impact of a fiber and vitamin B rich natural formula supplementing a low calorie balanced restrictive diet for reducing weight, on the course of obesity, by measuring both positive and negative influences exemplified in adiponectin, insulin hormones and homocysteine respectively.

SUBJECTS AND METHODS

Subjects

Twenty eight obese women participated in this study which lasted eight weeks. They were recruited from the National Research centre, Cairo Egypt as volunteers. The protocol for the study was approved by the National Research Center Ethics Committee. In addition, a written informed consent was obtained from each participant to be included in the study. The subjects involved in the study had a mean age of 47.86±2.18 years, and mean BMI of 34.10±0.95 kg/m². The study was divided into two phases of four weeks each. During the first phase (phase1), they followed a low calorie balanced diet, accompanied by the designed cookies, consumed as two cookies with breakfast and one with dinner to replace bread. The hypocaloric diet provided an average 48.3 g protein, 139 g carbohydrate, 29 g fat and 8 g fiber, which supply about 1000 kcal/day. The aim of the supplement was to enrich the hypocaloric regimen with a formula containing considerable amounts of vitamins, minerals and fiber producing a satiating effect. In addition, the highly extracted wheat flour contains betaine and choline, 747µg/g and 76µg/g, respectively [12]. In the second phase (phase 2), the same volunteers continued to follow only the same low calorie balanced diet, whereas the supplement was replaced by a portion of Egyptian bread that supplied equal amounts of calories, aiming to evaluate the health effect of the supplement on the same patients.

All individuals were subjected to thorough clinical examination at the beginning of the study. Relevant anthropometric measurements were recorded including weight, height and waist circumference using standard methods [13]. Body mass index (BMI) (weight in kg/height² in meter), fat free mass (FFM) and fat mass (FM) were calculated [14]. Blood pressure was measured, using a mercury sphygmomanometer.

Blood sampling and biochemical analysis

Fasting blood samples were obtained from the women before the dietary regimen at baseline, after the first 4 weeks (phase 1) and lastly at the end of the following 4 weeks (phase2). The blood samples were drawn in the morning after twelve hours fasting. Haemoglobin concentration was measured in the fresh samples by using the cyanomethaemoglobin method [15]. The rest of the blood samples were allowed to clot, centrifuged and sera separated. Blood glucose was determined in fresh sera using the oxide peroxidase method [16]. The remaining sera were divided into aliquots and stored in eppindorf tubes at –20 ^{o}C until used for further analysis. Serum total cholesterol, HDL-C and triglycerides were done using: cholesterol proceed No 1010, Stanbio HDL-C proceed No 0599, and stanbio Liquicolor triglycerides proceed No 2100, respectively. Friedewald formula was used to calculate LDL-C; LDL-C= [Total cholesterol] – [HDL-C] – [Triglyceride/5]. Serum Insulin was measured using Insulin Accu Bind ELISA Microwells Product Code: 2425-300 Monobind, INC,Costa Mesa, CA 92627 (USA) . Serum Adiponectin was measured using AviBion Human Adiponectin (Acrp30) ELISA Kit: ADIP025. Orgenium Laboratories. Viikinkaari 6. Fin- 00790 Helsinki FINLAND. Serum homocyteine measurement was done using

Axis Homocysteine EIA, distributed by IBL-Hamburg, Germany. IBL-Cat. - No.: AX 513 01.

Homeostatic model assessment (HOMA) was calculated using the equation:

Fasting insulin (µIu/ml**)** × [Fasting glucose (mg/dl) /405] [17].

Materials

The designed supplement formula in this study was prepared from highly extracted wheat flour (82%) and ground peanuts mixed in equal amounts. The formula was made into cookies by adding vegetable oil, yeast (Saccharomyces cerevisiae), water and cinnamon powder as a flavour. Each cookie weighed 20 g (Table 1).

Preparation of the cookie

Peanuts, whole wheat flour and the other ingredients were obtained from the local market (Cairo, Egypt). Peanuts were decorticated and then ground electrically in a mill (Wiley, model 4, England). The formula was made by mixing 460 g ground peanuts, 460 g whole wheat flour, 25 g corn oil, 25 g salt, 25g yeast and 5g cinnamon powder. Tap water (200 ml), was added and the blend kneaded using an electric mixer (Moulinex, France). The dough formed was allowed to ferment for 45 minutes, was cut into small pieces, put in cookie models and baked thermoelectrically in an oven at 180 ◦C for 20 minutes. After baking, the weight of each cookie was recorded, and each 3 were tightly wrapped and sealed in plastic food wrap.

Chemical analysis of the cookie supplement

Chemical analysis of the cookie was undertaken for macronutrients; including protein, fat and carbohydrate; using AOAC International methods: 668,931, 933 and 937, respectively [18]. Dietary fiber determination was done using AOAC International method: 985.29 [19]. Total energy was determined by calculation, taking in consideration that 3.6g crude fiber was included in the carbohydrate value, (protein energy= 42.4, carbohydrate energy=121.6, and fat energy=109.8). Micronutrients including: calcium, iron, magnesium, zinc and folate were calculated using World Food Dietary Assessment System (WFDAS), 1995, University of California, UNU/ FAO [20].

Statistical analysis

All values were expressed as mean± SE, two tailed student t-test was used to compare the two groups. Correlation between the different parameters was tested by Pearson test. P values <0.05 were considered statistically significant. SPSS window software version 17.0 (SPSS Inc. Chicago, IL, USA, 2008) was used.

RESULTS

Chemical composition of the cookie supplement

The data showed a high content of protein (10.6 g/100 g) and high dietary fiber content (6 g/100 g). The formula contains considerable amounts of folate (66.4 mg/100 g), magnesium (72 mg/100 g) and iron (2.3 mg/100 g) (Table 2).

Characteristic anthropometric parameters and blood pressure of the studied obese women during the two periods of dietary therapy

Comparison between the means of all the measured parameters showed a highly significant decrease ($P<0.01$) at the end of the two phases. Significant reduction in diastolic blood pressure (DBP) at $P<0.05$ was only recorded after the end of phase 1 (Table 3).

Haemoglobin and serum lipid profile

Significant increase was detected in the mean level of haemoglobin at the end of phase 1. Serum total cholesterol (T. cholesterol) concentration decreased by 5.28% and 3.37% after the end of phase 1, and phase 2, respectively. Significant elevation in the level of serum HDL-C (12.74%) was detected at the end of phase 1, while its level decreased significantly by 9.7% at the end of phase 2. The level of LDL-C and triglycerides decreased significantly at $p<0.05$ and at $p<0.01$ respectively, after the end of phase 1 only. Cardiovascular risk factor decreased significantly by (14.43%) in phase 1, while it increased significantly by (5.8%) at the end of phase 2, when compared to phase 1 (Table 4).

Serum glucose, insulin, adiponectin and homocysteine concentrations, and HOMA values

The mean values of all the parameters showed significant reduction ($P<0.01$) at the end of phase 1, while adiponectin hormone showed slight decrease by (1.3%). Homocysteine concentration increased significantly ($P<0.05$) at the end of phase 2, compared to phase 1. All other parameters showed slight numerical increase at the end of phase 2 (Table 5).

Relation between adiponectin, homocysteine, different biochemical parameters and blood pressure

Adiponectin showed a significant positive correlation coefficient at $P<0.01$ with the HDL-C concentration at all times of the investigations. Insulin, and HOMA negatively correlated with adiponectin, and the correlations were significant only at the start of the study. At the end of phase 2 the adiponectin level also correlated positively with the T. Cholesterol and fasting blood glucose. Homocysteine showed a significant positive correlation with cholesterol, LDL-C, and fasting glucose at baseline and with SBP, DBP and HOMA at the end of phase 1 (Table 6).

DISCUSSION

The discovery of adiponectin undoubtedly represents an important step to further understand the mechanism of obesity that induces insulin resistance and atherosclerosis [21]. Measurement of the adiponectin concentration in obese Egyptian women in this study showed that the mean serum concentration of the adiponectin ranged from 12.18±0.45 to 12.07±0.45 µg/ml, with little difference between the basal level and the other levels recorded after the two phases of dieting. This study was a short period type, yet the percent reduction in the body mass index (BMI) and fat mass (FM) values were satisfactory regarding the time of the experiment. At the end of the two dieting phases, however the women were still considered to be obese and still had relatively high BMI (33.2±0.89 versus 32.3±0.88 Kg/m^2) and FM (34.36±2.07 versus 32.78±1.44), which might be the reason for the little changes observed in the adiponectin concentration. In this respect, a recent study stated that serum adiponectin values displayed a threshold effect with increasing obesity beyond which concentration did not significantly decline [22]. In addition, another study found that adiponectin gene expression and plasma levels were not modified during calorie restriction [23]. However, the data in this study showed an intragroup variation in the adiponectin levels between the obese women before and during dieting. The range was 6.64 to 14.54 µg/ml at the basal level, 7.29 to 14.77 µg/ml at the end of the first phase, and 6.75 to 14.76 µg/ml at the end of the study. These results are in agreement with another study that reported marked variation in adiponectin concentrations even among obese subjects, whose levels ranged from 1.9 to 17 µg/ml [24]. As a result of this variation, adiponectin showed a positive correlation with HDL-C at the basal level and at the end of the two phases of dieting. In contrast, negative association were found between the adiponectin level, and each of insulin and HOMA, which may further support the concept of its protective role against arteriosclerosis and insulin resistance [4]. Our study could therefore suggest that since adiponectin is a natural hormone readily secreted by the body, and which actively participates in the web of controlling loops in an attempt to optimize the physiology of the internal environment towards normality, the small variations in the mean values reflect that the subjects were still obese, and that the positive correlations with HDL-C and the negative ones with insulin and HOMA would be conducive towards healthier outcomes.

Obesity affects blood lipids and homocysteine levels negatively. The early detection and control of obesity and the management of dyslipidemia and homocysteine levels may help to reduce the risk of cardiovascular disease in the young population [25]. Hyperhomocysteinemia promotes atherosclerosis and is most commonly carried out by B-vitamin deficiencies; especially folic acid, B_6 and $B_{12,}$ genetic disorders, certain drugs and renal impairment. Elevated homocysteine promotes atherosclerosis through increased oxidative stress, impaired endothelial function, and induction of thrombosis that increase the risk of cardiovascular disease by two folds and the risk of cerebrovascular disease to a lesser degree [26]. In addition, it has been reported that homocysteine thiolactone impairs insulin signalled by a mechanism involving oxidative stress, leading to a defect in insulin action [27]. The results of this study are in line with these reported data. A high mean for homocysteine level (17.3±0.94

µmol/L), accompanied by high values of both insulin concentration and the calculated HOMA value were found in the obese women. In the meantime, homocysteine showed a significant positive correlation with cholesterol, LDL-c and glucose concentration, which may increase the risk of atherosclerosis and diabetes mellitus. After the first phase of the dietary therapy, homocysteine concentration decreased significantly to reach 11.8±1.04 µmol/L which was accompanied by a significant decrease in the levels, and a significant positive correlation with insulin and HOMA values. In addition, it showed the same association with SBP and DBP. However in the second phase of dietary therapy, it was noticed that homocysteine, insulin and HOMA mean values increased significantly. Homocysteine mean value reached 13.4±1.04 µmol/L, yet optimal plasma homocysteine is defined as the level below 11.7µmol/L, and values above 12.0 µmol/L have consistently been associated with vascular disease [28]. The results obtained in this study could be explained by the protective high level of vitamin B in the peanut supplement used in the first period of the diet therapy. It has been demonstrated that the regular peanut consumers had a better quality diet -having higher contents of vitamin E, folate, magnesium, zinc and iron; and also that low B vitamins may follow a weight-reducing regimen [29]. Consequently, an increase in plasma homocysteine is anticipated and can be prevented by vitamin supplementation [30]. In addition, the high betaine content of the wheat used in the supplement formula may also have a lowering effect on the serum concentration of homocysteine. It has been reported that betaine is also effective in lowering homocysteine level [31].

CONCLUSION

The low calorie regimen supplemented by the weight reducing formula rich in dietary fiber, folate and betaine; and which has a low glycemic index proved to have a beneficial effect on body weight, serum levels of insulin and homocysteine; those play an important role in the process of the development of both atherosclerosis and insulin resistance. Equally important is that in spite of adiponectin hormone showing minor changes, its protective role against these two pathological conditions should be considered. This was a short term effect and a similar study should be recommended for longer period to ascertain the findings.

Table 1: Ingredients of the cookie supplement in grams (g/ Kg)

Ingredients	Amount (g/ kg)
Wheat flour	460
Ground peanut	460
Corn oil	25
Yeast	25
Salt	25
Cinnamon	5
Water (ml)	200

Table 2: Chemical composition of the supplement formula (/100 g)

Macronutrients	Amount	Micronutrients*	Amount
Energy (kcal)	274.0	Folate (mg)	66.4
Protein (g)	10.6	Calcium (mg)	32.8
Carbohydrate (g)	34	Magnesium (mg)	72
Fat (g)	12.2	Iron (mg)	2.3
Dietary fiber (g)	6.0	Zinc (mg)	1.6

***** World Food Dietary Assessment System (WFDAS), 1995, University of California, UNU/ FAO.

Table 3: Characteristic anthropometric parameters and blood pressure of the studied obese women during the two phases of dietary therapy

Parameters	Periods			% Change	
	Baseline	Phase1	Phase 2	Phase 1	Phase 2
Weight(kg)	83.70±1.8	81.50±1.65	79.50±1.61	-2.60**[a]	-2.50**[b]
BMI(kg/m^2)	34.10±0.95	33.20±0.89	32.30±0.88	-2.60**[a]	-2.70**[b]
FFM (kg)	47.68±0.42	47.12±0.38	46.74±0.27	-1.17**[a]	-0.81**[b]
FM (kg)	36.01±2.19	34.36±2.07	32.78±1.44	-4.58**[a]	-4.60**[b]
Waist(cm)	92.00±2.00	88.00±1.78	84.30±1.54	-4.40**[a]	-4.20**[b]
SBP(mmHg)	129.60±2.17	121.40±2.04	117.70±2.07	-6.30**[a]	-3.05**[b]
DBP(mmHg)	82.90±1.01	80.40±0.92	79.70±0.65	-3.00**[a]	-0.90

Values are expressed as mean± standard error (SE)

BMI: **B**ody **M**ass **I**ndex, **FFM**: **F**at **F**ree **M**ass, **FM**: **F**at **M**ass, **SBP**: **S**ystolic **B**lood **P**ressure, **DBP**: **D**iastolic **B**lood **P**ressure

** Significant at P≤0.01

[a] Baseline vs. phase 1

[b] Phase1 vs. Phase 2

Table 4: Haemoglobin and serum lipid profiles among obese women during the two phases of dietary therapy

Biochemical Parameters	Periods			% Change	
	Baseline	Phase 1	Phase 2	Phase 1	Phase 2
Haemoglobin(g/dl)	12.92±0.18	13.52±0.16	13.36±0.11	+4.46**[a]	-1.18
T.Cholesterol (mg/dl)	217.25±6.06	205.79±6.65	198.85±8.55	-5.28	-3.37
HDL-C(mg/dl)	55.12±2.00	62.14±2.43	56.11±2.39	+12.74**[a]	-9.7**[b]
LDL-C(mg/dl)	145.11±5.87	129.37±6.61	128.84±7.84	-10.85*[a]	-0.41
Triglycerides(mg/dl)	85.12±5.58	71.43±3.87	69.39±3.79	-16.08**[a]	-2.8
Risk factor (T.cholesterol/HDL-C)	4.02±0.14	3.44±0.16	3.64±0.17	-14.43**[a]	+5.8*[b]

Values are expressed as mean± standard error (SE)

T.Cholesterol: **T**otal **C**holesterol, **HDL-C**: **H**igh **D**ensity **L**ipoprotein-**C**holesterol, **LDL-C**: **L**ow **D**ensity **L**ipoprotein-**C**holesterol.

* Significant at P< 0.05 ** Significant at P<0.01

[a] Baseline vs. phase 1 [b] Phase1 vs. phase 2

Table 5: Fasting glucose, insulin, adiponectin hormones, homocysteine and HOMA values among obese women in the two phases of dietary therapy

Biochemical Parameters	Periods			% Change	
	Baseline	Phase 1	Phase 2	Phase 1	Phase 2
FBG (mg/dl)	96.52±1.54	86.37±1.21	87.16±2.19	-10.5**	+0.92
Insulin (µIU/ml)	14.37±1.69	8.61±0.66	9.56±0.76	-40.08**	+11.03
HOMA values	3.43±0.65	1.83±0.14	2.01±0.15	-41.65**	+9.84
Adiponectin (µg/ml)	12.18±0.45	12.01±0.44	12.07±0.45	-1.31	+0.50
Homocysteine (µmol/L)	17.30±0.94	11.80±1.04	13.4±1.01	-31.89**	+13.74*

Values are expressed as mean± standard error (SE)

FBG: Fasting **B**lood **G**lucose, **HOMA**: **H**omeostatic **M**odel **A**ssessment.

* Significant at P<0.05 ** Significant at P<0.01

Table 6: Correlation coefficient between adiponectin, homocysteine, and different biochemical parameters and blood pressure among obese women

Parameters	Adiponectin			Homocysteine		
	Periods					
	Basal	**Phase 1**	**Phase 2**	**Basal**	**Phase 1**	**Phase 2**
T.Cholesterol	0.313	0.348	0.406*	0.411*	-0.095	-0.044
LDL-C	0.048	0.119	0.203	0.461*	-0.351	-0.228
HDL-C	0.602**	0.574**	0.776**	-0.066	0.072	0.049
FBG	0.325	0.340	0.588**	0.582**	0.246	-0.017
Insulin	-0.569**	-0.179	-0.348	-0.062	0.364	0.337
HOMA	-0.533**	-0.138	-0.196	-0.020	0.414*	0.355
SBP	-0.148	0.272	0.352	0.121	0.531**	0.162
DBP	0.298	0.205	0.336	-0.012	0.510**	0.244

T.Cholesterol: **T**otal **C**holesterol, **LDL-C**: **L**ow **D**ensity **L**ipoprotein-**C**holesterol, **HDL-C**: **H**igh **D**ensity **L**ipoprotein-**C**holesterol, **FBG: F**asting **B**lood **G**lucose**, HOMA**: **H**omeostatic **M**odel **A**ssessment, **SBP**: **S**ystolic **B**lood **P**ressure, **DBP**: **D**iastolic **B**lood **P**ressure.

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

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