Ain Shams University
Faculty of Women for Arts, Science and Education
Biochemistry and Nutrition Department

Nutrigenomics and Gene Expression of Appetite Related Hormones in Relation with Adipocytokines in Obese Rats

Thesis

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بسم الله الرحمن الرحيم

"وَقُل رَبِ زِدني عِلماً"

صدق الله العظيم

سوره طه(114)

Dedication

This work is dedicated to my husband “Mostafa” and to my whole lovely family, especially my Father, Mother, brother and sisters.

Thanks for their love, encouragement, continuous help and support, I was always inspired to complete this work.

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Abstract

Nutrigenomics relates the resulting phenotypes of the major public health problem, obesity, to differences in the cellular and/or genetic response of the biological system following a nutritional stimulus. Obesity is characterized by successive production of pro-inflammatory adipocytokines and oxidative stress markers that negatively affects health. For that this study was conducted to evaluate the impact of different dietary components on gene expression of leptin and adiponectin in an attempt for understanding of how nutritional molecules affect metabolic pathways. Obesity was induced in adult male healthy Sprague-Dawley rats using high fat-high sucrose diet for 7.5 weeks. The animals were divided into 5 groups; 2 groups served as control groups and the other 3 groups fed the high fiber and/or high antioxidant vitamins (A & E) diets. The study involved measurement of the gene expression of leptin and adiponectin in adipose tissue. In addition of measuring energy intake, BMI and Lee index, some apparent digestibility measurements, as well as some biochemical measurements; adipocytokines (leptin, adiponectin, omentin, vaspin, TNF-α, IL-6), antioxidants (GSH, SOD, GPx), pro-oxidants (MDA, NO), blood lipids profile and insulin, HOMA-IR, QUICKI, glucose as well as HbA1c. The results indicated that dietary induction of obesity resulted in significant ($P<0.05$) increase in body weight, BMI, and Lee index by 40.9%, 40.7% and 11.6% respectively compared with normal rats. Obesity also significantly ($P<0.05$) increased leptin gene expression by 57.3% and decreased adiponectin expression level by 35.1% in adipose tissue compared with normal group. Serum leptin resistance, oxidative stress, pro-inflammation, lipotoxicity, and insulin resistance were induced by induction of obesity. However, treatment of obese rats with the mixed high fiber-high antioxidant vitamins diet significantly ($P<0.05$) reduced adipose tissue gene expression of leptin by 27.86% and its serum level by 30.34% and increased adiponectin expression level by 41.84% and its serum level by 99.09% compared with obese rats. Significant ($P<0.05$) reduction was also found in body weight, BMI and Lee index by 22.5%, 21.7% and 7.95% respectively compared with obese group. The anti-inflammatory cytokines omentin and vaspin were also significantly ($P<0.05$) increased by 20.2% and 180.6%, along with reduction in pro-inflammatory cytokines by treatment with the mixed diet compared with obese group. Oxidative stress status was reduced through reduction in the pro-oxidants and increment in the antioxidants and enzymes activities as compared with obese group. Blood lipids profile, atherogenic indices and insulin resistance conditions were also improved by the mixed diet as compared with obese group. Significant ($P<0.05$) increment in the serum level of HDL-C by 26.76% and reduction in LDL-C by 26.23% was found. Furthermore, significant ($P<0.05$) reduction in the values of HOMA-IR by 18.15% and increment in QUICKI by 2.59% was also observed in the treated group with the mixed diet as compared with the control obese group. In conclusion; the tested diets containing high fiber and antioxidant vitamins diet (A & E) could have a positive regulating effects on gene expression of some obesity related genes and thus improving the obesity and related risk conditions. Also, the tow newly discovered adipokines; omentin and vaspin, can be used as important serum markers of obesity related metabolic dysregulation.

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To

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Dear Dr. Huda Mohamad Ismail,

We are pleased to inform that your manuscript (no. 2016/IJBCRR/30489) entitled "**Impact of Different Dietary Components on Gene Expression of Leptin and Adiponectin in Adipose Tissue of Obese Rats**" is ACCEPTED for publication in **International Journal of Biochemistry Research & Review** (IJBCRR).

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Impact of Different Dietary Components on Gene Expression of Leptin and Adiponectin in Adipose Tissue of Obese Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author MAS designed the study and wrote the protocol. Authors SFD and HMIAEF performed the gene expression measurements. Author HMIAEF wrote the first draft of the manuscript. Authors AAER and HMIAEF managed and performed the biological experiment and analyses of the study. Author HMIAEF managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This study was conducted to evaluate the impact of different dietary components on gene expression of leptin and adiponectin to understand how nutritional molecules affect gene response and some metabolic pathways in diet-induced obesity in rats.
Methodology: Obesity was induced in adult male Spargue-Dawley rats using high fat- high sucrose diet for 7.5 weeks. The animals were divided into 5 groups; 2 groups served as control groups and the other 3 groups treated with the high fiber and/or high antioxidant vitamins (A & E)

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diets. The study involved measurement of leptin and adiponectin gene expression in adipose tissue using PCR technique. In addition, measurement of energy intake, Lee index, serum adipokines (adipocytokines) and some oxidative stress markers.

Results: The results indicated that dietary induction of obesity resulted in significant (P<0.05) increase in body weight and Lee index compared with normal rats. Obesity caused significant (P<0.05) elevation in leptin mRNA level by 57.3% and reduction in adiponectin mRNA level by 35.1% in adipose tissue compared with normal group. However, treatment of obese rats with high fiber and antioxidant vitamins (A & E) diet caused significant (P<0.05) reduction in adipose tissue gene expression of leptin by 27.86% with increased adiponectin mRNA level by 41.84%. A significant (P<0.05) reduction was found in body weight and Lee index by 22.5% and 7.95% respectively compared with control obese rats. Also serum anti-inflammatory cytokines omentin and vaspin as well as antioxidants were increased, with reduction in pro-inflammatory cytokines as well as malondialdehyde and nitric oxide significantly (P<0.05) as compared with their levels in obese rats before treatment.

Conclusion: Dietary modification through reducing caloric intake and/or increasing antioxidant vitamins (A & E) may have some effect on the adipose tissue mRNA levels of leptin and adiponectin as well as adipocytokine serum levels related to obesity condition.

Keywords: Adipokines; obesity; nutrigenomics; anthropometric measurements; gene expression; PCR.

1. INTRODUCTION

Obesity is a public health problem and its propagation is increasing globally. It is defined as extensive fat accumulation that impairs organ functions and negatively affects structures that may negatively affect health [1]. Human obesity may be genetic with environmental based causes, such as excessive consumption of high calorie foods in addition to sedentary lifestyle and reduced energy expenditure. These interactions might be reflected on gene expression. Obesity may involve changes in the expression of many genes that are thought to subject to multiple genetic/epigenetic controls and may have potential to modify the body's adipose tissue and glucose homeostasis [2]. Obesity might be associated with increased incidence of type 2 diabetes mellitus, cardiovascular diseases (CVD), hypertension, dyslipidemia and some cancers [3-4].

Adipose tissue is no longer considered as an inert tissue functioning as an energy store, but it is now considered as an endocrine organ critical for regulating metabolism in both health and disease states as it synthesize and release various bioactive molecules [5]. Of these are adipokines including adiponectin, leptin, omentin, vaspin, tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) [6]. Leptin, the gene product of the obesity gene, is directly associated with the regulation of adipose tissue mass and body weight through modulation of appetite and energy expenditure. Adiponectin plays a major role in glucose metabolism in insulin sensitive tissues [7]. Omentin and vaspin are proteins expressed and secreted from visceral adipose tissue that function to increase insulin sensitivity in adipose tissue [6,8]. During obesity adipokines production was found to be dysregulated. This condition promotes a low-grade inflammation in adipose tissue that may contribute to the pathogenesis of metabolic syndrome [9].

Oxidative stress is highly correlated with inflammatory and metabolic disease states, including obesity. It is highly correlated with accumulated damage in the body done by free radicals which adversely affect cell survival due to membrane damage through oxidative damage of lipid, protein and the irreversible DNA modification. Lipid peroxidation such as thiobarbituric acid reactive substances, malondialdehyde (MDA) and hydroperoxides are markers of oxidative damage of reactive oxygen species (ROS) [10].

Dietary fiber is not digested in the human intestine and it entraps the organic molecules (e.g. glucose), which play a critical role in controlling obesity and diabetes [11]. Besides, plant-derived dietary fibers were also enriched in phytochemicals which can be considered as dietary antioxidants. Consumption of diets rich in dietary fiber may help to reduce the incidence of common diseases such as obesity and diabetes [12]. Natural protection against oxidative stress can be provided by endogenous enzymes that capture free radicals such as superoxide dismutase or catalase and by nonenzymatic dietary compounds such as vitamins A and E [13]. ROS overproduction causes an imbalance between oxidative and antioxidative markers leading to abrogated oxidative stress. Obesity and metabolic syndrome were found to be characterized by oxidative stress and by reduced vitamin A and E blood levels suggesting that supplementation of diets with these antioxidant vitamins could reverse oxidative stress and the related risk factors [13,14].

The present work aimed to evaluate the impact of different dietary components on some appetite related hormones gene expression in adipose tissue of obese rats as a diet/gene and health interaction. And also the impact on blood levels of some oxidative stress markers, antioxidants and adipokines in comparison with healthy and obese rats.

2. MATERIALS AND METHODS

2.1 Animals

The experimental animals used throughout the work were normal adult male albino rats Spargue-Dawley strain weighing 200 ± 10 g supplied by the Breading Unit of the Egyptian Organization for Biological Products and Vaccines (Helwan, Egypt).

2.2 Diets

Diets were prepared on the basis of the balanced diet according to American Institute of Nutrition (AIN-93) which is adjusted by Reeves et al. [15]. The diets used in the study were: (1) Normal balanced diet, (2) High fat-high sucrose diet containing 21% fat and 55% carbohydrates (37% sucrose and 18% starch) according to Yang et al. [16] for induction of obesity, (3) High fiber low calorie diet (containing 20% fiber) according to Bragado et al. [17], (4) High antioxidant vitamins diet (containing 782 mg/kg diet vitamin A and 2.19 g/kg diet vitamin E) according to Soliman et al. [18] and (5) High fiber-high antioxidant vitamins diet (containing 20% fiber and 782 mg/kg diet vitamin A and 2.19 g/kg diet vitamin E).

2.3 Animal Trial

All rats were offered the balanced diet with drinking water ad libitum for 7 days for adaptation. Obesity was induced in rats by consumption of high fat-high sucrose diet for 7.5 weeks until measurement of obesity by lee index according to Auguet et al. [6].

$$\text{Lee index (g/cm)} = \frac{\sqrt[3]{\text{body}}\text{weight (g)}}{\text{Nose to anus length (cm)}}$$

Then the animals were divided into 5 groups as following:

- Group (1): Normal control rats consumed the balanced diet,
- Group (2): Obese control rats consumed the high fat-high sucrose diet,
- Group (3): Obese rats consumed high fiber diet,
- Group (4): Obese rats consumed high antioxidant vitamins (A & E) diet,
- Group (5): Obese rats consumed high fiber and high antioxidant vitamins (A & E) diet.

The experimental period was 4 weeks after induction of obesity during which constant weight of diets was given for each animal while water provided ad libitum. The gross energy of the diets and the energy intake of animals were calculated based on the conversion factors (protein 4.0, carbohydrates 3.74 and fat/oil 9.0) according to Owuamanam et al. [19]. The length in cm (from nose to anus) and weight of animals in grams was measured weekly to monitor the body weight changes and measuring of lee index [5]. All the measurements were done in anaesthetized rats by inhalation of diethyl ether [20]. At the end of experimental period all rats were euthanized under ether anesthesia after 12 hrs fasting with water ad libitum and blood was collected for whole blood, RBCs and serum measurements. The study was done in the animal's house of faculty of women for arts, science and education at Ain Shams University.

2.4 Collection of Adipose Tissue Samples

Visceral adipose tissue samples were collected and washed by 0.9% sterile sodium chloride solution. Tissue samples of 0.3 g were collected from the inside of the tissue using UV sterilized scissors and forceps. All samples were immediately frozen in liquid nitrogen. Then, all samples were stored at -80℃ [21] until they were used for the measurement of leptin and adiponectin gene expression using the polymerase chain reaction (PCR) technique.

Table 1. The sequences of primers for leptin, adiponectin and HPRT1 genes

Gene		Primers
Leptin	Forward sequence	5'TCACACACGCAGTCGGTATCC 3'
	Reverse sequence	5'GTCTCGCAGGTTCTCCAGGTC 3'
Adiponectin	Forward sequence	5'GCCGTTCTCTTCACCTACGA 3'
	Reverse sequence	5'CAGACTTGGTCTCCCACCTC 3'
HPRT1	Forward sequence	5'CTCATGGACTGATTATGGACAGGAC 3'
	Reverse sequence	5'GCAGGTCAGCAAAGAACTTATAGCC 3'

2.5 Gene Expression Measurements

Measurement of the gene expression of some appetite related hormones as leptin and adiponectin and the control housekeeping gene Hypoxanthine-guanine phosphoribosyl-transferase (*HPRT1)* in adipose tissue of normal and obese rats was done using the polymerase chain reaction (PCR) technique. It involved first the total RNA extraction from adipose tissue. Total RNA was extracted from adipose tissue using TRIzol total RNA extraction reagent following the methodology of TRIzol kit [22,23] (from life technologies company, CAT No. 15596-026). RNA was then converted to complementary DNA (cDNA) strands by reverse transcription using the sensiFast cDNA synthesis kit, (CAT. No. BIO-65053). Polymerase chain reaction (PCR) was applied using the Thermo Scientific Dream Taq Green PCR Master Mix (2X) (CAT. No. K1081). It is a ready-to-use solution containing Dream Taq DNA polymerase, optimized Dream Taq Green buffer, $MgCl_2$ and dNTPs. The master mix is supplemented with two tracking dyes and a density reagent that allows for direct loading of the PCR product on a gel. PCR product was loaded on 2% gel electrophoresis to separate and visualize the amplified cDNA strands according to their size using the method described by Yılmaz et al. [24]. All procedures were performed according to the manufacturers' protocols. Sequences for the selected primers are presented in the following Table 1 (above).

2.6 Biochemical Measurements

Serum omentin was measured using the ELISA kit (CAT. No. E0607Ra). While serum vaspin was measured using the ELISA kit (CAT. No. MBS260514). Serum interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) assay were performed according to the methods described by Gaines Das and Poole and D'Haens [25,26] respectively. The determination of GSH content in fresh EDTA whole blood was carried out using the colorimetric assay kit (CAT. No. GR2511) [27]. Erythrocyte superoxide dismutase (SOD) enzyme activity was determined according to the method described by Winterbourne et al. [28]. Glutathione peroxidase enzyme activity in RBCs was determined using the assay kit (CAT. No. GP2524) [29]. Malondialdehyde (MDA), the decomposition product from the peroxidation of polyunsaturated fatty acids, was measured in serum as thiobarbituric acid reactive substances according to Draper and Hadley [30]. And finally serum nitric oxide (NO) was determined according to the method of Montgomery and Dymock [31].

2.7 Statistical Analysis

Data were statistically analyzed by Statistical Package for Social Science (SPSS) version 20.0 statistical packages. Values were presented as mean ± standard deviation (S.D.). Statistical differences between groups were performed using one way ANOVA, the mean difference was significant at the (P<0.05) level according to Levesque [32].

3. RESULTS

3.1 Nutritional and Anthropometric Parameters

Our findings presented a significant (P<0.05) increase in the energy intake and Lee index up to 29.4% and 11.6% in obese rats fed the high fat-high sucrose diet compared with normal rats. However significant (P<0.05) reduction was found in the energy intake and Lee index reached 35.1% and 7.95% when obese rats were treated with diet rich in fiber and high antioxidant vitamins (A & E) compared with obese control group. Also significant (P<0.05) reduction was found in the values of Lee index (0.301±0.01 vs. 0.327±0.007) in high fiber diet group and (0.308±0.007 vs. 0.327±0.007) in the high antioxidant vitamins diet group vs. obese control group which may be caused by the reduced energy intake in these groups (Table 2 and Fig. 1).