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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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Original Research Article

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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].

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

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).

Table 2. The impact of different dietary components on some nutritional and anthropometric parameters in normal and obese rats

Groups	Energy intake (kcal/day)	Lee index (g/cm)
Group (1)	61.32±5.55[b]	0.293±0.006 [d]
Group (2)	79.34±5.75[a]	0.327±0.007 [a]
Group (3)	51.15±2.32[c]	0.301±0.01 [c]
Group (4)	61.66±4.62[b]	0.308±0.007 [b]
Group (5)	51.46±2.03[c]	0.303±0.007 [bc]

There was no significant difference between means have the same superscript letter in the same column. But results were significant (P<0.05) to each other for mean±SD bearing different superscript letter in the same column

3.2 The Gene Expression of Leptin and Adiponectin Genes in Adipose Tissues

Significant (*P*<0.05) elevation in leptin mRNA level by 57.3% and reduction in adiponectin mRNA level by 35.1% was observed in the adipose tissue of control obese rats compared with normal group. But treatment of obese rats with high fiber and high antioxidant vitamins (A & E) diet resulted in significant (*P*<0.05) reduction in leptin gene expression up to 27.86% with elevation in adiponectin gene expression up to 41.84% compared with control obese group. However neither the high fiber diet nor the high antioxidant vitamins diet consumed by obese rats showed significant (*P*<0.05) change in leptin and adiponectin gene expression compared with the control groups (Table 3 and Fig. 2).

3.3 Oxidative Stress and Antioxidant Parameters

A significant (*P*<0.05) increase was found in the pro-oxidative stress markers; MDA and NO serum levels (21.41±1.85 µmol/L vs. 2.14±0.09 µmol/L and 37.19±0.84 µmol/L vs. 17.96±0.52 µmol/L) in obese rats vs. normal rats respectively. Also significant (*P*<0.05) reduction in the blood antioxidant markers GSH level, SOD activity and GPx activity was found in obese rats by 63.8%, 55.77% and 29.28% compared with normal rats respectively. Whereas consumption of high fiber and high antioxidant vitamins (A & E) diet by obese rats resulted in significant (*P*<0.05) reduction in MDA and NO serum levels by 59.7% and 32.2% with increment in GSH levels, SOD activity and GPx activity by 101.2%, 94.02% and 28.81% compared with the control obese rats respectively. Treatment of obese rats with either high fiber diet or high antioxidant vitamins (A & E) diet showed significant (*P*<0.05) improvement in the oxidative stress condition observed by consumption of high fat-high sucrose diet. This appeared as significant (*P*<0.05) reduction in MDA and NO serum levels and increment in blood activities of SOD and GPx enzymes as well as GSH levels (Table 4 and Fig. 3-a and 3-b).

Table 3. The impact of different dietary components on the gene expression of leptin and adiponectin in normal and obese rats

Groups	Leptin gene expression	Adiponectin gene expression
Group (1)	0.89±0.06[b]	1.51±0.34[a]
Group (2)	1.40±0.33[a]	0.98±0.12[b]
Group (3)	1.22±0.33[ab]	1.27±0.15[ab]
Group (4)	1.07±0.22[ab]	1.24±0.13[ab]
Group (5)	1.01±0.22[b]	1.39±0.31[a]

There was no significant difference between means have the same superscript letter in the same column. But results were significant (P<0.05) to each other for mean±SD bearing different superscript letter in the same column

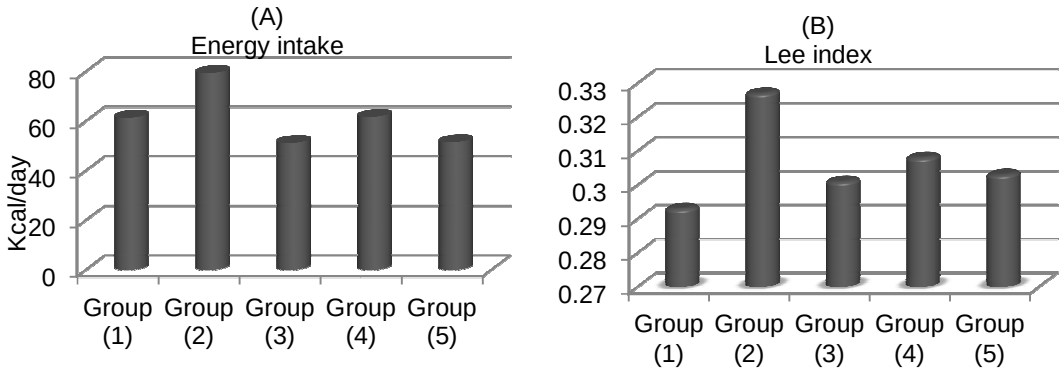

Fig. 1. Impact of different dietary components on (A) energy intake and (B) Lee index

3.4 Pro-inflammatory and Anti-inflammatory Adipokines

Obesity resulted in significant (P<0.05) increase in the pro-inflammatory adipokines, TNF-α and IL-6 serum levels by about 229.7% and 345.6% with concomitant reduction in the anti-inflammatory adipokines omentin and vaspin serum levels by 26.9% and 73.3% compared with normal rats respectively. Whereas treatment of obese rats with either high fiber diet or high antioxidant vitamins diet resulted in significant (P<0.05) decrease in serum levels of TNF-α (126.91±1.72 and 125.47±5.49 respectively) and IL-6 (17.57±0.61 and 16.07±0.44 respectively) and significant (P<0.05) increase in the serum levels of omentin (239.6±5.69 and 238.5±4.76 respectively) and vaspin (2.02±0.13 and

Table 4. The impact of different dietary components on some oxidative stress and antioxidant markers in normal and obese rats

Groups	Antioxidant markers			Oxidative markers	
	GSH (mg/dl)	SOD (U/ml)	GPx (U/ml)	MDA (µmol/L)	NO (µmol/L)
Group (1)	30.42±2.19[a]	422.00±7.82[a]	1250.4±68.5[a]	2.14±0.09[e]	17.96±0.52[e]
Group (2)	11.00±0.69[e]	186.67±12.96[e]	884.25±41.75[e]	21.41±1.85[a]	37.19±0.84[a]
Group (3)	16.33±1.29[d]	304.67±7.67[d]	1032.9±6.5[d]	14.17±1.10[b]	30.69±1.42[b]
Group (4)	19.45±1.74[c]	341.50±12.04[c]	1108.5±13.13[c]	10.58±1.11[c]	26.13±0.80[c]
Group (5)	22.13±1.61[b]	362.17±12.37[b]	1139.0±28.35[b]	8.63±0.89[d]	25.21±1.00[d]

There was no significant difference between means have the same superscript letter in the same column. But results were significant (P<0.05) to each other for mean±SD bearing different superscript letter in the same column

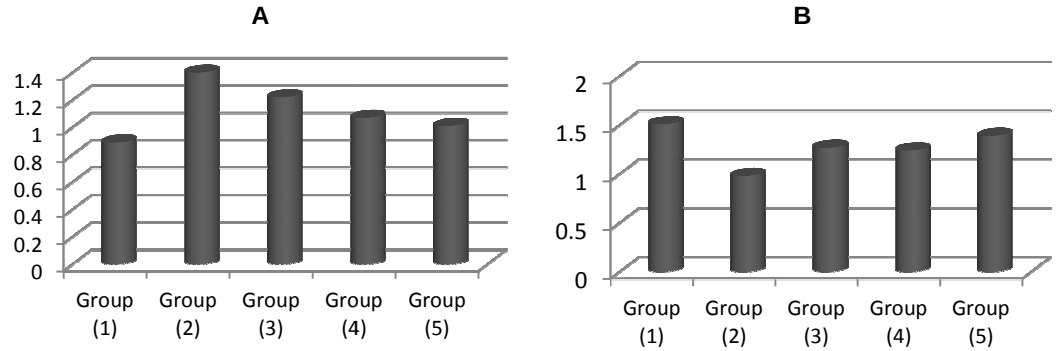

Fig. 2. Impact of different dietary components on (A) Leptin gene expression and (B) adiponectin gene expression levels in adipose tissue

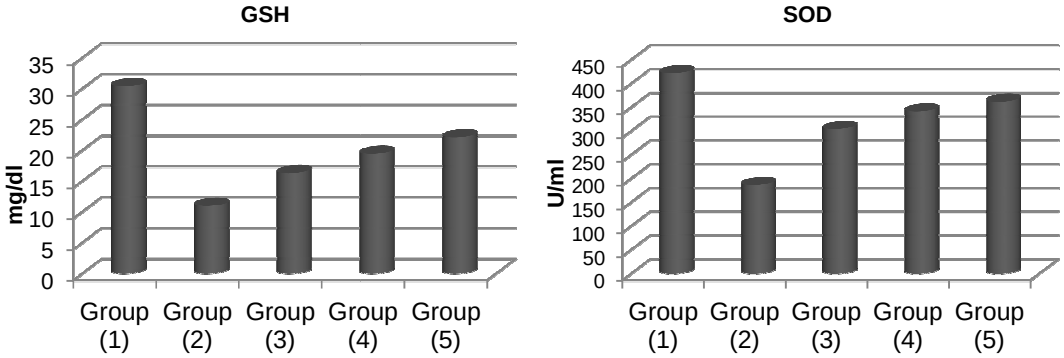

Fig. 3a. Impact of different dietary components on blood levels of GSH and SOD enzyme activity

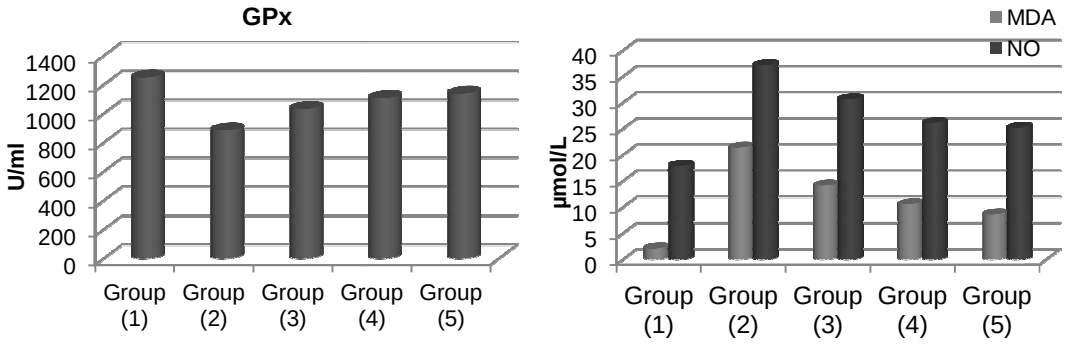

Fig. 3b. Impact of different dietary components on GPx enzyme activity as well as MDA and NO serum levels

1.80±0.05) compared with obese control group respectively. However, better results were found with combined treatment of obese rats with high fiber and high antioxidant vitamins (A & E) diet which caused significant (P<0.05) reduction in TNF-α and IL-6 serum levels by about 63.7% and 34.3% and increase in serum omentin and vaspin levels by 20.2% and 180.6% compared with obese rats respectively (Table 5 and Fig. 4).

4. DISCUSSION

Obesity might result from an energy imbalance attributable to our lifestyle, which includes physical inactivity and excessive intake of foods rich in saturated and trans-fats and refined sugars [33]. Obesity in rats can be easily estimated by Lee index. Alterations in Lee index were found to be associated with dyslipidemia and oxidative stress and may predict adverse effects of obesity. Body weight change and Lee index can be significantly higher as the calorie content of diets was higher than the normal diet [16,34,35]. Thus consumption of diets rich in fat and refined sugars can have potential to increase adipocyte size and body fat mass with development of metabolic alterations including oxidative stress, hyperlipidemia and insulin resistance [36].

As adipocytes become hypertrophied, their secretary profile evolves toward a more pro-inflammatory phenotype, where visceral adipose tissues showed greater secretion of inflammatory cytokines than subcutaneous adipocytes [37]. Adipocyte size of visceral adipose tissue have been found to correlate positively with lee index and serum leptin level, and negatively with serum adiponectin, and their mRNA expression in adipose tissue [37].

White adipose tissue is a source of some pro-inflammatory cytokines, where our study showed increased serum levels of TNF-α and IL-6 concomitant with adipose tissue accumulation in obese rats induced by feeding high fat-high sucrose diet. This can be explained by the fact that in obesity, hypertrophied white adipose tissue is infiltrated by macrophages, which may locally produce pro-inflammatory cytokines. This increase proportionally to adipocyte hypertrophy, lee index, BMI and body fat mass, and this effect is reversible on weight loss [38]. The excessive production of leptin by hypertrophied adipose tissue was found to regulate inflammation leading to increased release of cytokines such as IL-2 and TNF-α which is a pro-inflammatory response [39-41].

Our findings in obese rats indicated decreased serum levels of omentin and vaspin concomitant with consumption of high calorie diet. Serum omentin and vaspin levels were thus found to be negatively correlated with lee index and leptin and positively related to adiponectin levels [42,9]. Omentin was found to have an anti-inflammatory activity through decreased TNF-α-induced (NF-κB)- inflammatory signaling pathways [43]. Thus it may affect cytokine gene expression levels in adipose tissue with inhibition of TNF-α induced inflammation, which may in part lead to increased adiponectin mRNA [44]. Also vaspin has a compensatory role in the pro-inflammatory complications of obesity [45]. Vaspin was found to play a key role in human insulin resistance and lipid metabolism [42,45]. For that the reduced serum levels of omentin and vaspin in diet induced-obese rats may partially leads to increased pro-inflammatory condition and reduced expression of adiponectin gene in adipose tissue.

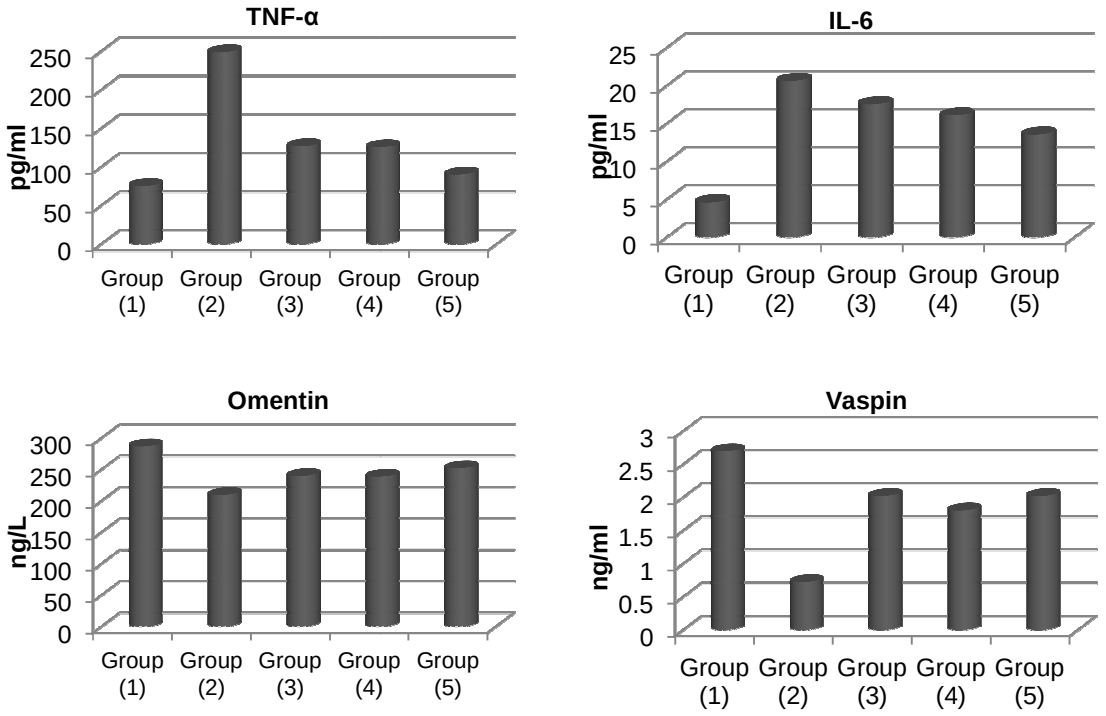

Fig. 4. Impact of different dietary components on serum levels of TNF-α, IL-6, omentin and vaspin

Table 5. The impact of different dietary components on some pro- and anti-inflammatory biomarkers in normal and obese rats

Groups	Anti-inflammatory		Pro-inflammatory	
	Omentin (ng/L)	Vaspin (ng/ml)	TNF-α (pg/ml)	IL-6 (pg/ml)
Group (1)	286.66±10.08^{a}	2.69±0.16^{a}	75.44±1.83^{d}	4.63±0.31^{e}
Group (2)	209.43±14.05^{d}	0.72±0.04^{c}	248.69±6.45^{a}	20.63±1.29^{a}
Group (3)	239.6±5.69^{c}	2.02±0.13^{b}	126.91±1.72^{b}	17.57±0.61^{b}
Group (4)	238.5±4.76^{c}	1.80±0.05^{b}	125.47±5.49^{b}	16.07±0.44^{c}
Group (5)	251.8±8.71^{b}	2.02±0.17^{b}	90.20±2.25^{c}	13.56±0.47^{d}

There was no significant difference between means have the same superscript letter in the same column. But results were significant (P<0.05) to each other for mean±SD bearing different superscript letter in the same column

Obesity-induced inflammation is frequently associated with increased oxidative stress [46] as observed by our findings through increased pro-oxidant markers as MDA and NO, with reduced enzymatic and non-enzymatic antioxidants. Pro-inflammatory mediators such as IL-6, leptin and TNF-α may induce the production of ROS generating a process known as oxidative stress [47]. Diets rich in lipid are also capable of generating ROS because they may alter oxygen metabolism [46,48].

Diminished antioxidant enzyme activities with increased adipose tissue accumulation in obese rats may be an important cause of oxidative stress in obesity [39]. Also the mitochondrial and peroxisomal oxidation reactions of fatty acids which produce ROS [49,50] and the over-consumption of oxygen in the mitochondrial respiratory chain which is coupled with oxidative phosphorylation in mitochondria and generate free radicals may be a proposed mechanism [39]. In oxidative stress status, the byproducts of lipid peroxidation are potent chemoattractants. Also ROS were found to augment the mRNA expression of NADPH oxidase in adipocytes with increased generation of ROS and may lead to increased pro-inflammatory changes in adipose

tissue. Thus, oxidative stress may result in the development of a vicious cycle that induces increased inflammation in adipose tissue [50,51]. This may in part explain the changes observed in the leptin and adiponectin genes expression profiles in adipose tissue of obese rats consumed the high fat- high sucrose diet.

For that, overweight and obesity can induce changes in body composition with dysregulation of leptin and adiponectin genes expression [52,53]. Our study showed that feeding animals with high fat-high sucrose diet resulted in induction of obesity with an increase in leptin gene expression and decrease in adiponectin gene expression. The levels of leptin mRNA in adipocytes are greatly correlated with intracellular lipid content [54], as leptin promoter contains lipid sensing mechanisms that regulate leptin expression in response to changes in the amount of intracellular lipid [55]. Also the observed adipocyte hypertrophy may induce tissue hypoxia which is another stressor in the adipose tissues of obese patients, when fat mass rapidly expands more than the capabilities of its blood supply. This may affect the expression of leptin and adiponectin genes in obesity [56]. In diet induced-obesity, the increased pro-inflammatory cytokines such as TNF-α and IL-6 might be able to suppress adiponectin gene expression [57,58]. Also mitochondrial dysfunction-induced ROS overproduction may be associated with decreased levels of adiponectin in hypertrophied adipose tissues [59].

On the other hand, treatment of obese rats with high fiber diets showed marked improvement in the deteriorated effects caused by the high fat-high sucrose diet. It has been reported that at least a 10% reduction in body weight was necessary to reverse the pro-inflammatory parameters which contribute to oxidative stress during obesity [60]. In comparison with small adipocytes, larger adipocytes secrete higher levels of pro-inflammatory markers. Thus a calorie restriction feeding regimen induced marked increase in lipid mobilization leading to reduction in adipocyte cell size and lowered cytoplasmic lipid content [61]. Consumption of foods rich in fiber were linked to enhanced satiety and reduced energy intake, and therefore plays an important role in weight regulation and reduced risk of disease [62,63]. For that dietary fibers can be used to control obesity [64,65].

The ability of dietary fibers to decrease body weight could be contributed to several factors including; induced satiety, decreased energy intake, decreased fat digestibility, reduced metabolizable energy of diet and decreased intake of simple carbohydrates [66,65]. As weight loss in obese patients can improve the inflammatory condition of visceral adipose tissue, dietary fibers, which reduce body weight, may indirectly reduce leptin gene expression and increase adiponectin gene expression thus reducing inflammation. Supplementation of obese rats with dietary fiber may reduce body weight and subsequently reduce circulating TNF-α and IL-6 levels [67,68]. Thus the hypocaloric diet-induced weight loss can be accompanied by improvement of the anti-inflammatory profile and increased serum omentin levels [69,9]. The weight loss was also accompanied by decreased MDA and leptin serum levels [70]. High fiber diets may also increase the antioxidant capacity of the antioxidants in the serum of obese treated rats [67,71].

Antioxidant dietary nutrients may have protective effects on oxidative stress conditions. Obese persons have lower serum levels of antioxidants than normal persons, which mean that antioxidants in obesity are consumed more than in normal-weight individuals [48]. Consumption of fruits and vegetables rich in carotenoids can protect against obesity and its related health risks such as cancer and cardiovascular diseases [72].

Plasma concentrations of carotenoids and vitamin E were found to be lower in adult subjects with BMI ≥25 kg/m^2 compared to those who had lower BMI <25 kg/m^2. Levels of carotenoids and α-tocopherol were found to be negatively correlated with body weight, lee index, BMI and all measures of general and central obesity [35,48,73,74]. Chronic dietary vitamin A supplementation was found to increase the thermogenic activity in brown adipose tissue and muscle thus reduce body fat content, and oppose the development of obesity [75]. Retinol and α-tocopherol may reduce inflammatory reactions by inhibiting the production of pro-inflammatory cytokines, as well as neutralizing free radicals [76].

The observed reduction in pro-inflammatory cytokines and oxidative stress by vitamin supplementation can be described in part by the fact that vitamin A promotes differentiation towards more anti-inflammatory cytokines relative to the pro-inflammatory cytokines production [74]. Vitamin E was also found to inhibit signaling pathways, such as the family of

MAPK. These MAPK, which was activated by ROS, modulates the release of pro-inflammatory cytokines [77,78]. This suggested the role of vitamins A and E, in controlling the inflammatory condition in adipocytes [77,79]. Vitamin A and E administration to obese rats normalized changes in MDA levels and serum antioxidant enzyme activity in obese rats [35,17]. Vitamin E has a very extensive function of protecting biological membrane in human body and nucleic acids in cells from damage by free radicals. It can directly remove O^2-, quench singlet oxygen and superoxide dismutase and establish an antioxidant system in human body together with glutathione peroxidase. Vitamin E plays its antioxidant activity through the reaction with lipid oxygen radicals and lipid peroxyl free radicals, by providing protons to break lipid peroxidation chain reaction [80,81]. It also inhibits the activity of ROS-generating systems such as iNOS or NADPH oxidase and reduced oxidative stress in diabetic and obese mice [82].

This modulation of inflammatory and oxidative stress conditions by antioxidant vitamins supplementation may in part leads to changes in the gene expression levels of leptin and adiponectin in adipose tissue. Vitamin A has potential to modulate adipokines gene expression [83]. Retinoic acid has the ability to downregulate leptin mRNA expression in white adipose tissue. This suggests the negative transcription regulation of leptin gene by vitamin A and its metabolites [84]. Also vitamin E may regulate the expression of adipokines such as leptin and adiponectin and decrease oxidative stress in obesity [6,85]. The observed augmentation in the expression of the leptin gene in the WAT from rats fed a high fat-high sucrose diet in our study was down regulated by vitamins A and E supplementation, and this may be as a result of the activation of the transcription factor - peroxisome proliferator activated receptor gamma (PPARγ) by vitamin E which can induce the expression of adiponectin gene and reduce leptin gene expression levels [86].

5. CONCLUSION

High fat- high sucrose diet can have great effect on inducing excessive fat accumulation and increasing the risk of developing obesity. Diets rich in saturated fat and refined sugars resulted in increased expression of leptin gene and reduced expression of adiponectin gene in adipose tissue with induced pro-inflammatory condition and oxidative stress status development. However a shift of diet towards lower calorie content and/or higher antioxidant vitamins (A & E) content could have significant improvement in the obesity condition with reduction in animal's weights and the obesity related risk conditions as well as reduction in the leptin and increased adiponectin genes expression. This resulted in a shift of the inflammatory condition towards more anti-inflammatory profile and reduced pro-oxidative status with enhanced antioxidant defense mechanisms. The modulation effects occurred in the gene expression levels of leptin and adiponectin in adipose tissue of obese rats treated with dietary fiber alone may be attributed to the effect of dietary fiber on reducing body weight and adipocyte cells size. However the inclusion of antioxidant vitamins (A & E) with dietary fiber improved inflammatory and oxidative profiles leading to modulation of gene expression profiles. Combination diets containing higher fiber and antioxidant vitamins (A & E) intake may simply have more protective effects, as a diet/gene and health interaction, against development of obesity and its related risk diseases than either diet alone.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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