

Original Research Article

Nutrigenomics; Impact of Different Nutritional Treatments on the Gene Expression of Leptin and Adiponectin in Adipose Tissue of Obese Rats

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 the field of nutrigenomics in case of obesity. **Methodology:** Obesity was induced in adult male Spargue-Dawley rats using high fat and sucrose diet for 7.5 weeks. The animals were divided into 5 groups; 2 groups served as control groups and the other 3 groups as treatments which were fed the high fiber and/or high antioxidant vitamins (A & E) diets. The study involved measuring the gene expression of leptin and adiponectin in adipose tissue using PCR technique. In addition of measuring energy intake, Lee index, serum adipokines 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 diet caused significant ($P<0.05$) reduction in adipose 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 anti-inflammatory cytokines omentin and vaspin and serum 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:** The field of nutrigenomics can be useful tool in the improvement of obesity and related risk conditions and prevention of developing other diseases as diabetes mellitus and cardiovascular diseases.

Key words: Adipokines, Obesity, Nutrigenomics, Anthropometric measurements, gene expression, PCR.

1. INTRODUCTION:

Nutritional genomics was described as the field that may improve dietary guidelines for human disease prevention and health [1]. It includes nutrigenetics and nutrigenomics. Nutrigenomics explores the effects of dietary components on the gene expression, DNA methylation patterns, proteome and metabolome [2]. The concept of nutrigenomics builds on the facts that: 1) diet and dietary components can modulate multiple processes involved with onset, progression, and severity of diseases so that altering the risk of disease development; 2) food components can act, either directly or indirectly, on the human genome to alter the expression of genes and gene products; 3) diet could compensate for the effects of genetic polymorphisms; and 4) the consequences of dietary components are dependent on an individual's genetic background and on the balance of health and disease states [3].

Obesity is a public health problem and its propagation is increasing globally. It is defined as extensive fat accumulation that impaired organs functions and negatively affected structures that may negatively affects health. According to the WHO, obesity was defined as BMI ≥ 30 kg/m² [4]. 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. It was found that the risk of excessive weight gain in children of obese parents was increased from two- to three-times for moderate obesity and up to eight times for severe obesity [5]. Obesity might be associated with increased incidence of type 2 diabetes mellitus, cardiovascular diseases (CVD), hypertension, dyslipidemia and some cancers [6]. Furthermore, obesity may involve changes in the expression of many genes that are thought to subject to multiple genetic/epigenetic controls. Cytokine/chemokine receptor genes, pro/anti-inflammatory genes, regulators of white adipose tissue

(WAT), components of the insulin receptor complex, as well as transcription factors, and the miRNAs that regulate their expression; have potential to modify the body's adipose tissue and glucose homeostasis [7].

Oxidative stress is highly correlated with inflammatory and metabolic disease states, including obesity. Also it is highly correlated with accumulated damage in the body done by free radicals which were inadequately neutralized by antioxidants. It has been found that free radicals 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). It was suggested that obesity result in a cluster of sources of oxidative stress including; increased tissue lipid levels, hyperglycemia, increased rates of free radical formation, inadequate antioxidant defenses, and chronic inflammation [8]. Adipokines (adipocytokines) are the cytokines produced by adipocytes. 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 synthesizes and release various bioactive molecules [9]. Of these adipokines are adiponectin, leptin, omentin, vaspin, tumor necrosis factor alpha (TNF- α), interleukin-6 (IL-6) and interleukin-1 (IL-1) [10]. Omentin and vaspin are proteins expressed and secreted from visceral adipose tissue that function to increase insulin sensitivity in adipose tissue [10,11]. During obesity some adipokines as leptin, TNF- α , and IL-6 are overproduced, whereas adiponectin and omentin plasma levels were decreased. This condition promotes a low-grade inflammation in adipose tissue that may contribute to the pathogenesis of metabolic syndrome, which is a cluster of metabolic abnormalities characterized by central obesity, dyslipidemia, insulin resistance, type-2 diabetes mellitus and cardiovascular diseases [12].

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 [13]. After entering the large intestine, it is fermented and provides colonic bacteria by carbohydrates and produces short chain fatty acids that help to maintain the colonic health. Besides, plant-derived dietary fibers were also enriched in phytochemicals which can be considered as dietary antioxidants. Thus, the consumption of diet rich in dietary fiber helps to reduce the incidence of common diseases such as diabetes, obesity and colon cancer [14]. Further Vargas-Robles and coworkers discussed that 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, E and C [15]. ROS overproduction causes an imbalance between oxidative and antioxidative markers leading to abrogated oxidative stress. Obesity and metabolic syndrome were characterized by oxidative stress and by reduced vitamin A, E and C blood levels suggesting that supplementation of diets with these antioxidant vitamins could reverse oxidative stress and the related risk factors [15,16].

The present work aimed to evaluate the diet/gene health interaction in obesity through the nutrigenomics and the effect of different dietary treatments on some appetite related hormones gene expression, in adipose tissue of adult male obese rats in comparison with healthy rats, also the relation of oxidative stress and the inflammatory adipokines, which can be used as biomarkers of diagnostic value.

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. It was supplied by the Breeding 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 prepared according to American Institute of Nutrition (AIN-93) which is adjusted by Reeves (1993) [17]. 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 [18] for induction of obesity, (3) High fiber low calorie diet (containing 20% fiber) according to [19], (4) High antioxidant vitamins diet (containing 782 mg/kg diet vitamin A and 2.19 g/kg diet vitamin E) according to [20], (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 [8].

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

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 were given for each rat 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 [21]. 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 [8]. All the measurements were done in anaesthetized rats by inhalation of diethyl ether [22]. At the end of experimental period all rats were sacrificed under ether anesthesia after 12 hrs fasting with water ad libitum and blood was collected for whole blood, RBCs and serum measurements.

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 sterile scissors and forceps. All samples were immediately frozen in liquid nitrogen. Then, all samples were stored at -80°C [23] until they were used for the measurement of leptin and adiponectin gene expression using the polymerase chain reaction (PCR) technique.

2.5. Gene expression measurements:

Genetic investigation 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 method described by [24] and [25] (from life technologies company, CAT No. 15596-026). RNA was converted in to complementary DNA (cDNA) strand by reverse transcription using the sensiFast cDNA synthesis kit, (CAT. No. BIO-65053). Polymerase chain reaction (PCR) was applied using the Thermo Scientific DreamTaq 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, MgCl₂ 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 [26]. The sequences for the selected primers were presented in the following table:

Gene	primers	
Leptin	Forward sequence	5'TCACACACGCAGTCGGTATCC 3'
	Reverse sequence	5'GTCTCGCAGGTTCTCCAGGTC 3'
Adiponectin	Forward sequence	5'GCCGTTCTCTTCACCTACGA 3'
	Reverse sequence	5'CAGACTTGGTCTCCACCTC 3'
HPRT1	Forward sequence	5'CTCATGGACTGATTATGGACAGGAC 3'
	Reverse sequence	5'GCAGGTCAGCAAAGAACTTATAGCC 3'

2.6. Biochemical measurements:

Serum omentin measurement was performed 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 [27] and [28] respectively. The determination of GSH content in fresh EDTA whole blood was carried out according to the method described by [29] using the colorimetric assay kit (CAT. No. GR2511). Erythrocyte superoxide dismutase (SOD) enzyme activity

was determined according to the method described by [30]. Glutathione peroxidase enzyme activity in RBCs was determined according to the method of [31] using the assay kit (CAT. No. GP2524). Malondialdehyde (MDA), the decomposition product from the peroxidation of polyunsaturated fatty acids, was measured in serum as thiobarbituric acid reactive substances according to [32]. And finally nitric oxide (NO) was determined in serum according to the method of [33].

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 $\pm$ 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 [34].

3. RESULTS

3.1. Nutritional and anthropometric parameters:

Our findings presented a significant ($P<0.05$) increase 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 in the energy intake and Lee index reached 35.1% and 7.95% when obese rats were treated with diet rich in fiber and antioxidant vitamins (A & E) (Table 1).

Table (1): The impact of different dietary treatments on some nutritional and anthropometric parameters in normal and obese rats

Groups	Energy intake (kcal/day)	Lee index (g/cm)
Normal group (negative control)	61.32 $\pm$ 5.55 ^b	0.293 $\pm$ 0.006 ^d
Obese group (positive control)	79.34 $\pm$ 5.75 ^a	0.327 $\pm$ 0.007 ^a
Obese rats consumed high fiber diet	51.15 $\pm$ 2.32 ^c	0.301 $\pm$ 0.01 ^c
Obese rats consumed high antioxidant vitamins (A & E) diet	61.66 $\pm$ 4.62 ^b	0.308 $\pm$ 0.007 ^b
Obese rats consumed high fiber & antioxidant vitamins (A & E) diet	51.46 $\pm$ 2.03 ^c	0.303 $\pm$ 0.007 ^{bc}

There was no significant difference between means have the same letter in the same column ($P<0.05$).

3.2. The gene expression of leptin and adiponectin genes in adipose tissues:

The data presented significant ($P<0.05$) elevation in leptin mRNA level by 57.3% and reduction in adiponectin mRNA level by 35.1% in adipose tissue of the control obese rats as compared with normal group. But treatment of obese rats with high fiber and 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 (Table 2).

Table (2): 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
Normal group(negative control)	0.89 $\pm$ 0.06 ^b	1.51 $\pm$ 0.34 ^a
Obese group(positive control)	1.40 $\pm$ 0.33 ^a	0.98 $\pm$ 0.12 ^b
Obese rats consumed high fiber diet	1.22 $\pm$ 0.33 ^{ab}	1.27 $\pm$ 0.15 ^{ab}
Obese rats consumed high antioxidant vitamins (A & E) diet	1.07 $\pm$ 0.22 ^{ab}	1.24 $\pm$ 0.13 ^{ab}
Obese rats consumed high fiber & antioxidant vitamins (A & E) diet	1.01 $\pm$ 0.22 ^b	1.39 $\pm$ 0.31 ^a

There was no significant difference between means have the same letter in the same column ($P<0.05$).

3.3. Oxidative stress and antioxidant parameters:

A significant ($P<0.05$) increase were found in the pro-oxidative stress markers; MDA and NO serum levels in obese rats about 21.41 ± 1.85 $\mu\text{mol/L}$ vs. 2.14 ± 0.09 $\mu\text{mol/L}$ and 37.19 ± 0.84 $\mu\text{mol/L}$ vs. 17.96 ± 0.52 $\mu\text{mol/L}$ as compared with normal rats respectively. While significant ($P<0.05$) reductions in the antioxidant markers GSH levels and SOD and GPx activities were found in obese rats by 63.8%, 55.77% and 29.28% as compared with normal rats respectively. Whereas consumption of high fiber and antioxidant vitamins diet by obese rats resulted in significant ($P<0.05$) reduction in MDA and NO serum levels by 59.7% and 32.2% and increment in GSH levels and SOD and GPx activities by 101.2%, 94.02% and 28.81% as compared with the control obese rats respectively (Table 3).

Table (3): The impact of different dietary treatments 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 ($\mu\text{mol/L}$)	NO ($\mu\text{mol/L}$)
Normal group(negative control)	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
Obese group(positive control)	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
Obese rats consumed high fiber diet	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
Obese rats consumed high antioxidant vitamins (A & E) diet	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
Obese rats consumed high fiber & antioxidant vitamins (A & E) diet	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 letter in the same column ($P<0.05$).

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% as compared with control normal group respectively. Whereas treatment of obese rats with high fiber diet resulted in significant ($P<0.05$) decrease in TNF- α and IL-6 serum levels by about 48.97% and 14.8% and increase in the levels of omentin and vaspin by 14.4 % and 180.6% as compared with obese control group respectively. However, better results were found with combined treatment of obese rats with high fiber and 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% respectively (Table 4).

Table (4): The impact of different dietary treatments 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)
Normal group(negative control)	286.66 ± 10.08^a	2.69 ± 0.16^a	75.44 ± 1.83^d	4.63 ± 0.31^e
Obese group(positive control)	209.43 ± 14.05^d	0.72 ± 0.04^c	248.69 ± 6.45^a	20.63 ± 1.29^a
Obese rats consumed high fiber diet	239.6 ± 5.69^c	2.02 ± 0.13^b	126.91 ± 1.72^b	17.57 ± 0.61^b

Obese rats consumed high antioxidant vitamins (A & E) diet	238.5±4.76 ^c	1.80±0.05 ^b	125.47±5.49 ^b	16.07±0.44 ^c
Obese rats consumed high fiber & antioxidant vitamins (A & E) diet	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 letter in the same column ($P<0.05$).

4. DISCUSSION

Nutrigenomics explains the effects of dietary nutrients on the gene expression, DNA methylation patterns, proteome and metabolome [1]. It clarifies how the DNA and genetic code affect our needs for certain nutrients and within specific quantities and helps to maintain optimal health throughout life. It also refers to the application of genomic factors in nutritional research, and enables us to formulate links between specific nutrients and genetic principles, e.g. the way in which food ingredients influence gene expression. Nutrigenomics facilitate understanding of how nutrition can affect metabolic pathways and how these processes are related to diet-related diseases [35, 36]. 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. It has been reported that consumption of a high-fat diet and reduced physical activity are potential triggers of chronic public diseases such as obesity, type 2 diabetes mellitus and cardiovascular diseases [37]. Adipocytes secrete various bioactive proteins called adipokines, such as leptin and adiponectin, into the blood stream. Leptin, the gene product of the obesity gene, is directly associated with the regulation of adipose tissue mass and body weight in humans and rodents through modulation of appetite and energy expenditure. Adiponectin plays a major role in glucose metabolism in insulin sensitive tissues in both humans and animals [38].

Obesity can be easily estimated from the Lee index in rats and alterations in Lee index were associated with dyslipidemia and oxidative stress in serum of rats and may predict the adverse alterations of obesity. It has been found that Lee index was significantly higher in sucrose fed and high CHO diet fed adult Wistar rats, after 30 days of treatment, than in the control group [39]. Also the weight of mice fed with high fat diet during 12 weeks was found to be significantly higher than weights of normal mice [40]. Similarly; mice fed on high fat-high sucrose (HF-HS) diet gained adipose tissue and body weights more rapidly as compared with those fed on standard diet over 4 weeks period [18]. Thus consumption of diets rich in fats can have potential to increase body fat mass with development of metabolic alterations including hyperlipidemia, impaired glucose tolerance and insulin resistance [41]. In accordance, as adipocytes become hypertrophied, their secretory profile evolves toward a more pro-inflammatory phenotype, where visceral adipose tissues showed greater secretion of inflammatory cytokines than subcutaneous adipocytes. Adipocyte size of visceral adipose tissue have been found to correlate positively with lee index, insulin resistance, and serum leptin, and negatively with serum adiponectin, and their mRNA expression in adipose tissue [42]. For that, overweight and obesity can induce changes in body composition with dysregulation of leptin and adiponectin gene expression. As feeding animals on high fat diet (HFD) resulted in increased leptin and decreased adiponectin gene expressions in WAT [43]. Boque and coworkers found that gene expression levels of leptin were upregulated by high fat and sucrose diet (HFS) [44].

This can be described by different mechanisms. Leptin promoter methylation can be modulated by different factors such as high-fat diet and impaired glucose tolerance [45]. Changes in plasma leptin levels can be associated with changes in leptin mRNA levels per adipocyte and the levels of leptin mRNA in adipocyte are greatly correlated with intracellular lipid content [46], as leptin promoter contains lipid sensing mechanisms that regulate leptin expression in response to changes in the amount of intracellular lipid [47]. Tissue hypoxia is another stressor in the adipose tissues of obese patients, when fat mass rapidly expands more than the capabilities of its blood supply. In this situation, Hypoxia-inducible transcription factor-1 (HIF-1 α) is induced, that binds to leptin promoter and lead to transactivation of leptin gene expression [48]. Leptin promoter DNA methylation in adipose tissue underlies an epigenetic mechanism regulating leptin expression [49]. It has been found that high fat and sucrose diet resulted in hypermethylation in two CpG sites of leptin promoter [44]. Hypermethylation at CpG sites 6–7 and 29–30 and a hypomethylation of CpG site 15 of leptin gene promoter were found. However, the shift to a chow diet after exposure to the obesogenic diet reverted these results, particularly in CpG sites 29–30 [49]. The mechanisms by which obesity decreased adiponectin expression might be that; in obesity, the adipose tissues exert anti-inflammatory effects against adipose tissue inflammation. Adiponectin secreted by adipocytes may accumulate in the stromal vascular fraction in interstitial space of inflamed obese adipose tissue, especially in the sites of macrophages. Globular adiponectin, acting via AdipoR1, can inhibit Toll-like receptor-mediated nuclear factor- κ B

(NF- κ B) signaling, and full-length adiponectin, via the AdipoR2, potentially shifted the polarization of macrophage cells to an M2 phenotype (anti-inflammatory) [50]. Pro-inflammatory cytokines such as TNF- α and IL-1 β were able to induce DNA methyltransferase-1 (DNMT-1) expression and activity, and inducing hypermethylation of the adiponectin promoter R2 (positioned 1 kb upstream of the transcription start site). NF- κ B signaling pathway also appeared to induce stimulation of DNMT-1[51]. In addition, TNF- α induced a closed chromatin structure in the adiponectin promoter and enhanced the recruitment of DNMT-1 and methyl CpG-binding protein 2 (MeCP2), which is a methyl-DNA-binding protein that can interact with histone-modifying enzymes to the R2 region. Simultaneously, the level of histone-3 (H3) acetylation at the R2 was decreased by TNF- α in adipocytes leading to suppression of adiponectin gene expression [51]. Also mitochondrial dysfunction-induced ROS overproduction was found to be associated with decreased levels of adiponectin in adipose tissues. Interestingly, mitochondrial dysfunction increases the endoplasmic reticulum stress (ER), providing a mechanistic relation between mitochondrial dysfunction, ER stress and adiponectin downregulation [52].

White adipose tissue is a source of some pro-inflammatory cytokines such as TNF- α and IL-6, which may have local and systemic effects. In obesity, WAT is infiltrated by macrophages, which may locally produce pro-inflammatory cytokines. Infiltration of macrophage in adipose tissue increases proportionally to adipocyte hypertrophy, BMI and body fat mass, and this effect is reversible on weight loss [53]. Leptin can regulate inflammation through T- lymphocytes proliferation and activation. Leptin influences T cells to release cytokines such as IL-2 and TNF- α , which is a pro-inflammatory Th-1 response that activate the innate immunity increasing IL-6 and TNF- α cytokines. Also the adhesion molecules on endothelium were found to be increased by leptin in obesity which leads to increased transmigration of monocytes and increased macrophages infiltration to white adipose tissue. Some of these macrophages fuse and create large multinucleated cells, in obesity, producing higher amounts of TNF- α , IL-6 and chemokines [54]. In obesity the increased pro-inflammatory mediators' expression are via NF- κ B or JNK. JNK bind and phosphorylate c-Jun (on Ser-63 and Ser-73) within its transcriptional activation domain and belongs to the mitogen-activated protein kinase (MAPK) family, responsive to stress stimuli such as cytokines [55]. This may in part explain the increased serum levels of IL-6 and TNF- α observed in obese patients than in the control healthy persons [56]. It has been found that omentin-1 affected cytokine gene expression levels in adipose tissue; increasing adiponectin mRNA and decreasing IL-6 and TNF- α mRNA levels with inhibition of TNF- α induced inflammation [57]. TNF- α stimulates the NF- κ B pathway, which in turn increases the expression of TNF- α and other pro-inflammatory adipokines. Omentin have an anti-inflammatory activity through decreased TNF- α -induced (NF- κ B)- inflammatory signaling pathways [58]. Vaspin plays a key role in human insulin resistance and lipid metabolism. It may be a compensatory cytokine in the pathogenesis of insulin resistance condition and obesity associated alterations [59]. It have been postulated that vaspin, acting as a serine protease inhibitor, can inhibit a protease which plays an important role in the degradation of molecules with direct or indirect lipid and glucose lowering effects. Vaspin expression and its serum levels were found to decrease as diabetes condition worsened [60]. On the other hand, the administration of vaspin in diet-induced obese mice improved insulin sensitivity and glucose tolerance, and reversed the expression of the genes that promote insulin resistance such as leptin, resistin and TNF- α . This suggested that vaspin has a compensatory role in the pro-inflammatory complications of obesity [60]. Serum omentin and vaspin levels were found to be significantly reduced in obese patients compared with healthy control persons. Serum omentin levels were found to be negatively correlated with BMI, insulin, LDL-cholesterol, TAG and leptin and positively related to HDL-cholesterol and adiponectin levels [59,11].

Obesity-induced inflammation is frequently associated with increased oxidative stress [61]. Pro-inflammatory mediators such as IL-6, leptin and TNF- α may induce the production of reactive oxygen species generating a process known as oxidative stress [62]. Diets rich in lipid are also capable of generating ROS because they may alter oxygen metabolism. Upon the increased expansion of adipose tissue, the activities of antioxidant enzymes such as GPx, SOD and CAT were found to be significantly diminished [61,63]. Adipose tissue was considered as an important source for the generation of systemic oxidative stress [64]. Obesity can produce oxidative stress via several mechanisms. The first, is the mitochondrial and peroxisomal oxidation reactions of fatty acids which produce ROS [65], the second mechanism, is the over-consumption of oxygen in the mitochondrial respiratory chain which is coupled with oxidative phosphorylation in mitochondria and generate free radicals. Third, diminished antioxidant enzymes activities with the increase of adipose tissue with subsequent tissue hypoxia [54]. It is well known that macrophages produce ROS, thus macrophages infiltration in obese adipose tissue were involved in increased ROS generation. 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 macrophage infiltration and 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 [65]. Singh and Singh observed a significant increase in the blood levels of

malondialdehyde (MDA) while significant decrement was recorded in the levels of superoxide dismutase, catalase, reduced glutathione (GSH), glutathione reductase (GR), and glutathione peroxidase (GPx) in obese men subjects in comparison to normal healthy men subjects. Their observations suggested that oxidative stress is induced in the obese subjects (BMI>46 kg/m²) and might be responsible for increased risk for diseases like diabetes, cancer, atherosclerosis etc. [66].

On the other hand [1] studies suggested that at least a 10 % reduction in body weight was necessary to reverse the pro-inflammatory parameters which contribute to oxidative stress during obesity. In comparison with small adipocytes, larger adipocytes secrete higher levels of pro-inflammatory markers that promote insulin resistance. Thus a calorie restriction feeding regimen induced marked increase in lipid mobilization leading to reduction in adipocyte cell size and lowered cytoplasmic lipid content [67]. Dietary fibers include naturally occurring edible carbohydrate polymers, have many physiological effects that are of benefits to health. The consumption of foods rich in fiber were linked to enhanced satiety and resulted in reduced energy intake, and therefore plays an important role in weight regulation and reduced risk of disease [68,69]. For that using dietary factor including dietary fiber can be used to control obesity. Increasing dietary fiber intake may decrease energy absorption by diluting a diet's energy availability [70,71]. The ability of dietary fibers to decrease body weight could be contributed to several factors. (1) Fermentation of soluble fiber in the large intestine, result in production of glucagon-like peptide (GLP-1) and peptide YY (PYY) which play a key role inducing satiety. (2) Dietary fiber may decrease energy intake. (3) Dietary fiber may decrease fat digestibility and thus reduced the metabolizable energy (ME) of diet, which is gross energy minus the energy lost in feces, urine and combustible gases. Finally, as dietary fiber intake increases, the intake of simple carbohydrates decreases [72,71]. As weight loss in obese patients can improve the inflammatory condition of visceral adipose tissue, dietary fibers, which reduce body weight, may indirectly reduce inflammation. It was found that supplementation of obese Zucker rats with dietary fiber reduced body weight and subsequently reduced circulating TNF- α and IL-6 levels. This lower inflammatory response might be attributed to the low glycemic impact of dietary fibers, which contribute to a lower inflammatory status [73,74]. Thus the hypocaloric diet-induced weight loss can be accompanied by improvement of insulin sensitivity and increased serum omentin levels [75,11]. Decreased caloric consumption caused significant weight loss in obese women. The weight loss was accompanied by decreased oxidative stress marker MDA, IL-6, TNF- α , TAG, adiponectin and leptin serum levels [76]. Similarly wheat bran- and barley-based diets tended to increase the antioxidant capacity of the antioxidants in the serum of obese treated rats. However, the increase in serum antioxidant capacity in wheat bran- and barley-fed obese rats did not reduce the amount of oxidized lipids in the plasma, as measured by MDA levels, or increase glutathione peroxidase activity [73,77]. In another study, obese rats fed the wheat bran diet had higher glutathione peroxidase enzyme activity and reduced plasma MDA than the control obese rats [73].

Hosseini and coworkers indicated that antioxidant dietary nutrients may have protective effects on oxidative stress conditions. Obese persons have lower serum levels of antioxidants than normal persons; it is also possible that higher oxidative stress in obese individuals might mean antioxidants are consumed more than in normal-weight individuals [63]. Consumption of fruits and vegetables rich in carotenoids protects against obesity and its related health risks such as cancer and cardiovascular diseases [78]. Plasma concentrations of carotenoids and vitamin E were found to be lower in adult subjects with BMI ≥ 25 kg/m² compared to those who had lower BMI <25 kg/m². Levels of carotenoids and α -tocopherol were negatively correlated with body weight, lee index, BMI and all measures of general and central obesity [63,79]. It has been observed that vitamin A or vitamin E supplementation induced significant higher weight loss in obese mice. Vitamin A enriched diet greatly improved lipids metabolism and development of obesity in young and adults obese rats [40,80]. For that, serum retinol and α -tocopherol levels have negative correlation of with the body weight of obese patients. Further, they reduced inflammatory reactions by inhibiting the production of pro-inflammatory cytokines in macrophages, as well as neutralized free radicals [81]. Chronic dietary vitamin A supplementation increased the thermogenic activity in brown adipose tissue and muscle thus reduced body fat content, and opposed the development of obesity. Vitamin A has potential to improve glucose tolerance and insulin sensitivity through modulation of adipokines gene expression [82]. Retinoic acid has potential to downregulate leptin mRNA expression in white adipose tissue. This suggests the negative transcription regulation of leptin gene by vitamin A and its metabolites [83]. Also vitamin E has been shown to regulate the expression of adipokines such as leptin and adiponectin and decrease oxidative stress in a high fat diet-induced obesity in rats [38]. Furthermore, the augmented expression of the leptin gene expression in the WAT from rats fed a high fat diet was downregulated by vitamin E supplementation within three-weeks [84]. The proposed association between vitamin E and leptin gene regulation in obesity may result from the activation of the transcription factor - peroxisome proliferator activated receptor gamma (PPAR γ) by vitamin E which can induce the expression of adiponectin gene but reduce the leptin gene expression levels [85]. On the other hand vitamin A promotes differentiation towards Th2

cells (the anti-inflammatory response) and increases the ratio of Th2 cytokines relative to Th1 cytokines. In obesity, vitamin A deficiency seems to increase the Th1 response, and participates in fat deposition and obesity associated chronic inflammatory process [80]. However; vitamin E supplementation was able to reduce the obesity-induced increases in leptin, IL-6 and TNF- α blood levels [86]. 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 [87]. This suggested the role of vitamin E, in controlling the inflammatory condition in adipocytes [86,88]. Soliman and coworkers found that vitamin A and E administration to obese rats normalized changes in MDA levels which were increased by induction of obesity. Moreover, serum antioxidant enzyme activity was decreased in obese rats and normalized in vitamin A and E administered obese rats [40,20] for more reviews. Vitamin E can increase mRNA expression level of CAT, SOD and GPx, and reduce oxidative stress indicators such as TBARS. 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 O²⁻, 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 [89,90]. It also has been shown to inhibit the activity of ROS-generating systems such as iNOS or NADPH oxidase and reduced oxidative stress in diabetic and obese mice [91].

5. CONCLUSION

In conclusion; high fat and sucrose diet can have great effect in inducing excessive fat accumulation and increased risk of developing obesity. Diets rich in saturated fat and refined carbohydrates resulted in increased expression of leptin gene in adipose tissue which increased pro-inflammatory condition and oxidative stress status development with reduction of the gene expression of adiponectin in adipose tissue and the levels of anti-inflammatory cytokines as well as antioxidants. However a shift of diet towards lower calorie content and higher antioxidant vitamins (A and E) content could have significant improvement in the obesity condition with reduction in animal's weights and the obesity related risk conditions including reduction in the leptin gene expression and increased adiponectin gene expression. This resulted in a shift of the inflammatory condition towards more anti-inflammatory condition and reduced pro-oxidative status with enhanced antioxidant defense systems. The modulation effects occurred in the gene expression levels of leptin and adiponectin in adipose tissue of obese rats may be attributed to the effect of dietary fiber and antioxidant vitamins on reducing body weight and adipocyte cells size in animals.

The field of nutrigenomics can be useful tool in the improvement of obesity and its related risk conditions and prevention of developing other diseases as diabetes mellitus and cardiovascular diseases.

ETHICAL APPROVAL

It is not applicable.

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