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Effects of High Vegetables Consumption on Inflammatory and Immune Functions in Human Obesity

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The aim of this study is to demonstrate the effect of consumption of certain vegetables soup as a source of antioxidant with a low caloric balanced regimen to reduce weight, on the defined inflammation and immune biomarkers. Thirty obese Egyptian women shared in this study which lasted for four weeks. They divided in two groups, group 1 had a mean age 40.26±2.01 years and body mass index (BMI) 35.13±1.06, they followed a low caloric balanced regimen (900-1000 kcal) accompanied with the designed vegetables soup. This soup composed of 30 g different dried vegetables chosen and added to each others according to their antioxidant and palatability contents, to be consumed half hour before lunch and dinner after boiling in water. Group 2 (control) had mean age 41.0±3.85 years and BMI 39.22±1.42, they followed the same hypocaloric balanced regimen only. Relevant anthropometric measurements were taken include weight, height and abdominal I circumference (minimal waist), BMI was calculated. Haemoglobin, blood sugar, total cholesterol (TC), triglyceride, serum zinc (Zn), copper (Cu) and magnesium (Mg) were determined. The serum leptin and interleukin-6(IL-6) as proinflammatory and inflammatory biomarkers were estimated beside the immunoglobulins IgA, IgG and IgM to represent a sort of immunity status. All parameters were done before and after 2 and 4 weeks of the intervention. At the end of the study the body weight, BMI and abdominal I circumference significantly decreased, the decrease in the abdominal I circumference was higher among group 1. Slight insignificant variation were observed in the levels of both haemoglobin and blood sugar. Serum TC was decreased significantly in both groups while serum triglyceride decreased only among group 1. The serum mineral Zn, Cu and Mg showed a sort of variation but all the figures were within the normal ranges. Significant decrease was observed in the serum leptin among the two groups, while insignificant decrease in the mean levels of serum IL-6 was detected among group I only. The three immunoglobulins IgA, IgG and IgM were improved significantly, IgA was significantly correlated with both IgG and IgM, these results were quite apparent in group 1. In conclusion obesity corresponds to a subclinical inflammatory condition with increase level of IL-6 and leptin, accompanied by immunity modulation. The uses of vegetables soup rich in the phenolic compounds as supplement, with the low caloric balanced regimen to reduce weight, proved to improve these effects.

Key words: Obesity, anthropometric measurements, interleukin-6, leptin, immunoglobulins

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INTRODUCTION

Epidemiologic evidence of this rising tide of obesity and associated pathologies has led in the last decade to a dramatic increase of research on the role of adipose tissue as an active participant in controlling the body physiologic and pathologic processes. The current view of adipose tissue is that of an active secretory organ sending out and responding to signals that modulate appetite, energy expenditure, insulin sensitivity, endocrine and reproductive systems, bone metabolism and inflammation and immunity (Fantuzzi, 2005). In this context obesity particularly visceral adiposity is associated with chronic low grade inflammation as indicated by increase level of inflammatory markers, the inflammatory cytokine interleukin-6 (IL-6) and the tumor necrosis factor α (TNF α) are expressed in human adipose tissue (Eckel *et al.*, 2002; Mohamed *et al.*, 1997). Inflammation is a source of oxidative stress, increase production of reactive oxygen species may also enhance the inflammatory response by activating redox-sensitive nuclear transcription factors. These transcription factors are essential for inducible immune and inflammatory responses, including cytokines cell adhesion molecules and inducible NO synthase (Lavrovsky *et al.*, 2000).

Adipokines are proteins produced mainly by adipocytes. Although adipose tissue secretes a variety of factors only leptin and adiponectin (and possibly resistin, adipsin and visfatin) are primary produced by adipocytes and can therefore be properly classified as adipokines (Fantuzzi, 2005). In addition to acting as a regulator of food intake and energy expenditure (Ahima and Flier, 2000), there is a growing evidence to suggest that leptin is a modulator of immune and proinflammatory responses (Fantuzz and Faggioni, 2000; Faggoni *et al.*, 2001). Leptin is an adipocyte-derived hormone/cytokine that links nutritional status with neuroendocrine and immune functions (8). Following the cloning of genes such as leptin (Ob), leptin receptor (ObR), pro-opiomelanocortin (POMC) and melanocorten-4 receptor (MC4-R), it has been found that certain genetic alteration (ie mutation), loss of function among others can cause obesity and also significantly affect immune responses (La Cava and Matarese, 2004). So it is now widely accepted that obesity (and also associated metabolic conditions) can have a huge impact on immune responses and therefore on the development of several pathologic conditions (Matarese and La Cuva, 2005). It has been reported that changes produced during obesity effect both humoral and cellular immunity (Munoz *et al.*, 2004). The impaired immunity may account for higher susceptibility to infection and certain types of cancers among obese (Tanaka *et al.*, 1998).

So if obesity is a condition of increase oxidative stress that accompanied by a low rate of chronic inflammation and alteration of immunity, obese individual may benefit from antioxidant supplementation used in combination with different regimens to reduce weight. The aim of this study is to demonstrate the effect of using dried vegetables soup as a source of antioxidant with a balanced low caloric regimen to reduce body weight and to demonstrate its effect on certain inflammatory and immune biomarkers among obese Egyptian women.

MATERIALS AND METHODS

Thirty obese Egyptian women aged 30-50 years were recruited from the National Research Centre as a volunteers. All subjects had a stable weight and were healthy according to medical history, clinical examination and routine laboratory finding. The women were divided to two groups; group 1 with mean BMI 35.13±1.06 was instructed to follow a low caloric balanced diet (1000 kcal) plus the vegetables mixture, while the second group (control) with mean BMI 39.22±1.42 followed only the low caloric balanced regimen. The study last for 4 weeks in which the patients were followed up every 2 weeks to estimate the effect of the vegetables on the body weight and the biochemical parameters, compared to the control group that was consuming the same food items of the hypocaloric diet. In the global strategy on diet, physical activity and health development by WHO, the increase of fruit and vegetable consumption is one of the recommendation (Anonymous, 2004).

Anthropometric measurements: Weight and height were recorded according to standard methods (Jelliffe, 1966). Abdominal circumference minimal waist was measured using flexible light metal tape. BMI were calculated according to: weight (in kg)/height^2 (in meter). The values were recorded before starting and at the mid and the end of the study.

Blood sampling and biochemical analysis: Blood samples were obtained from the women before starting the regimen (base) and after two weeks (mid) and at the end of the study (final). The sample was taken in the morning after 12 h fasting. Blood glucose was determined in fresh samples using oxide peroxide methods (Asatoor and King, 1980). Haemoglobin level was measured using cyanomethaemoglobin method (Van Kampen and Zijlstra, 1961). Blood was centrifuged and serum then was stored at -20°C until needed for further analysis. Serum total cholesterol and triglyceride were done using, respectively cholesterol procedure N0. 1010, triglycerides procedure No. 2100, Stanbio and Liquicolor Kit, Texas (Stein, 1986; Wahlefeld, 1974). Serum minerals including zinc, copper were determined using SENTINEL, CH Kit (determination of zinc, copper in serum without deproteinization, Cat.17.1255 and Cat 17.108, Germany) (Homsher and Zak, 1985; Abe *et al*., 1989). The diagnostics ELITECH Kit, Cat No MAGN-0600 was used for serum Mg determination, Spain (Elin, 1987). Serum leptin was done by using the DSL-10.23100, human leptin enzyme-linked immunosorbent (ELISA) Kit, Webster, Texas 77598-4217 USA (Guillaume and Bjorntorp, 1996). An immunoenzymometric assay for the quantitative measurement of human interleukin-6 (IL-6) in serum was done using Biosource IL-6 EASIA Kit Belgium (Maxey *et al*., 1992). Immunoglobulin determination (IgG, IgA, IgM) was carried out using BINDARID Kit Q RK002, Brimingham, B 29 6AT UK (Macini *et al*., 1965).

Materials: A natural vegetables mixture was prepared from different kinds of fresh vegetables. They were dried at low temperature (40°C) with circulating air and added to each other in certain amount taking in consideration their known antioxidant contents. Seven packages each containing 30 g from the dried vegetables mixture were given to every woman weekly to be prepared using boiling water to be eaten as a soup ½ h before lunch and dinner.

Chemical analysis: Chemical analysis of the dried vegetables mixture (Table 1) were undertaken for macronutrients including protein, fat and carbohydrate using the AOAC methods, 1990. The micronutrients including both vitamins and minerals were determined by using Liquid Chromatography (Chase *et al*., 1993; Simpson *et al*., 1985; Desia and Machlin, 1985) and atomic absorption apparatus (Hussein and Bruggeman, 1997), respectively. Polyphenols determination was done using Colorimetric method (Singleton and Rossi, 1965).

Table 1: Chemical analysis of the macro-and micronutrients of the dried dietary vegetables mixture used in the soup (100 g)

Nutrients	Values
Water (g/100 g)	12.24
Total protein (g/100 g)	12.98
Fat (g/100 g)	2.22
Ash (g/100 g)	7.80
Carbohydrate (g/100 g)	55.23
β-Carotene (μg)	9945.01
Vitamin E (mg)	6.64
Vitamin B_1 (μg/100 g)	0.40
Niacin (μg/100 g)	20.73
Calcium (mg/100 g)	326.25
Magnesium (mg/100 g)	225.91
Iron (mg/100 g)	25.10
Zinc (mg/100 g)	3.33
Potassium (mg/100 g)	2721.17
Sodium (mg/100 g)	351.92
Total phenolic (mg of gallic acid g^{-1} dry wt.)	10.76

Statistical analysis: Statistical significance was set at the $p<0.05$ and less and values are expressed as mean±SEM. Dependent t tests were calculated comparing all measured variables and pearson product moment correlations were used to test the relation between variables using SPSS programme, version 7.5.

RESULTS

Table 1 showed the chemical analysis of the dried vegetables mixture per 100 g dried weight include protein, fat and carbohydrate. The Table 1 showed the high content of the minerals and vitamins in the dried mixture and the total phenolic compound was quantitated.

The head of the cabbage, lettuce, radish and the beet root were added to reach 40% of the total amount. Other vegetables 60% were added depending on their antioxidant activity and the palatability of the mixture (Table 2).

High significant decrease was reported in all the anthropometric measurements at $p<0.05$ in both groups. The mean haemoglobin level of both groups showed slight variation at the three visits, women of group 1 were slightly anaemic. Glucose levels were at the normal range. TC levels decreased in both groups, significant difference was reported between the base and the mid visit at $p<0.05$ in group 1, while significant differences were detected between the different levels at the different visits in group 1. Serum triglyceride decrease in both groups but the decrease was insignificant (Table 3).

The mean concentration of serum Zn, Mg of the first groups increased by 10.74 and 12.02% at the mid and the end of the study, respectively, while the Cu concentration remain constant with slight variations. In group 1 the mean concentrations of the serum three minerals increased. After intervention the mean level of leptin decreased significantly in group 1 ($p<0.05$) and between both the mid and the final visits in group 2 at $p<0.05$-0.01.The mean serum concentration of the IL-6 in group I

Table 2: Percent portion of the different vegetables in the mixture

Vegetables	Percent
Cabbage head	10
Radish head	10
Lettuce head	10
Beet root	10
Sweet pepper	20
Onion	15
Tomato	15
Parsley	10

Table 3: Mean±SEM of age, anthropometric measurements and biochemical parameters of obese women before, during and after intervention

Parameters	Base	Mid	Final	First percent change	Final percent change
Group 1 (n = 22)					
Age (year)	40.26±2.01				
Height (cm)	156.36±1.02				
Weight (kg)	85.58±2.26	83.27±2.19**	81.90±2.15 ††,‡‡	-2.69	-4.30
BMI (kg m^{-2})	35.13±1.06	34.19±1.05**	33.64±1.03 ††,‡‡	-2.68	-4.24
Waist cir. (cm)	92.35±1.89	88.32±1.51**	86.72±1.48 ††,‡‡	-4.36	-6.09
Haemoglobin (g dL^{-1})	12.78±0.34	12.72±0.29	12.58±0.31	-0.47	-1.56
Blood sugar (mg 100 mL^{-1})	90.82±2.51	88.55±1.99	91.46±1.86	-2.49	+0.70
Cholesterol (mg dL^{-1})	217.57±9.45	200.59±8.89**	209.04±9.12	-7.80	-3.92
Triglyceride (mg dL^{-1})	100.62±10.08	101.02±13.06	84.04±7.93	+0.39	-16.48
Group 2 (control) (n = 8)					
Age (year)	41.00±3.85				
Height (cm)	152.80±1.02				
Weight (kg)	91.56±3.42	89.44±3.16**	87.25±3.22 ††,‡‡	-2.32	-4.71
BMI (kg m^{-2})	39.22±1.42	38.32±1.35**	37.39±1.39 ††,‡‡	-2.29	-4.67
Waist cir. (cm)	93.88±1.91	91.05±2.34**	89.88±2.36 ††,‡‡	-3.01	-4.26
Haemoglobin (g dL^{-1})	11.38±0.20	11.83±0.26	11.88±0.22	+3.95	+4.39
Blood sugar (mg 100 mL^{-1})	83.00±2.46	84.75±5.02	91.70±2.65	+2.11	+10.48
Cholesterol (mg dL^{-1})	198.53±15.43	177.13±17.48*	179.15±18.26†	-10.78	-9.76
Triglyceride (mg dL^{-1})	90.56±18.94	102.29±13.57	92.60±14.95	+12.95	+2.25

*,**: Significantly different between base and mid *: p<0.05, **: p<0.01. †,††: Significantly different between base and final †: p<0.05, ††: p<0.01. Significantly different between mid and final ‡: p<0.05, ‡‡: p<0.01

Table 4: Mean±SEM of minerals, leptin, interleukin-6 and immunoglobulins of obese women before, during and after intervention

Minerals concentrations	Base	Mid	Final	First percent change	Final percent change
Group 1 (n = 22)					
Zinc (µg dL^{-1})	87.11±6.19	89.53±5.87	96.47±5.31	+2.78	+10.74
Copper (µg dL^{-1})	126.26±8.15	125.66±7.70	121.29±7.34	-0.48	-3.94
Magnesium (mg dL^{-1})	2.33±0.05	2.44±0.10	2.61±0.15	+4.72	+12.02
Leptin (ng mL^{-1})	93.19±9.35	79.25±11.03	74.07±9.99†	-14.96	-20.52
Interleukin-6 (pg mL^{-1})	15.90±1.56	13.53±1.12	13.06±1.33	-14.90	-17.86
IgA (mg L^{-1})	2313.95±222.34	2548.10±187.68	3144.12±330.27††,‡‡	+10.12	+35.88
IgG (mg L^{-1})	9549.05±916.56	10929.52±695.45**	12250.59±892.40 †	+14.46	+28.29
IgM (mg L^{-1})	1316.38±126.52	1507.48±96.70**	1687.18±122.77 ††,‡‡	+14.52	+28.17
Group 2 (control) (n = 8)					
Zinc (µg dL^{-1})	84.37±7.13	98.94±9.29**	108.25±5.51 ††	+17.27	+28.30
Copper (µg dL^{-1})	123.89±13.09	152.39±7.98	125.68±3.24 ‡‡	+23.00	+1.44
Magnesium (mg dL^{-1})	2.36±0.03	2.32±0.12	2.62±0.03 ††	-1.69	+11.02
Leptin (ng mL^{-1})	104.81±7.33	92.34±9.65**	88.81±10.51 ††,‡	-11.89	-15.27
Interleukin-6 (pg mL^{-1})	17.90±2.83	17.93±0.97	19.98±3.99	+0.17	+11.62
IgA (mg L^{-1})	1900.00±144.13	2018.13±58.20	2308.75±53.99 ††,‡‡	+6.22	+21.51
IgG (mg L^{-1})	7809.50±591.40	8626.87±349.94	9928.50±69.77 ††,‡‡	+10.47	+27.13
IgM (mg L^{-1})	1224.13±58.86	1366.50±4.29*	1455.00±10.31†,‡‡	+11.63	+18.86

Significantly different between base and mid *: p<0.05, **: p<0.01. Significantly different between base and final †: p<0.05, ††: p<0.01. Significantly different between mid and final ‡: p<0.05, ‡‡: p<0.01

Table 5: Correlation coefficient between obesity indices and both serum leptin and IL-6 levels among obese women at the different periods of intervention

	Group 1 n = 22						Group 2 n = 8					
Anthropometric and	Leptin			IL-6			Leptin			IL-6		
biochemistry	Base	Mid	Final	Base	Mid	Final	Base	Mid	Final	Base	Mid	Final
Weight	0.570**	0.645**	0.674**	NS	NS	NS	0.876**	0.879*	0.951**	NS	NS	NS
BMI	0.505*	0.657**	0.634**	0.531*	NS	NS	0.932***	0.932*	0.971**	0.971*	NS	NS

*: p<0.05, **: p<0.01

decreased by -14.96 and -17.86% in the mid and final visit, respectively, while no change and 11.62% increase was detected among women of group 1 at the same visits. The mean serum concentration of the three immunoglobulins IgA, LgG and IgM increased significantly after intervention (Table 4).

Table 5 showed significant positive correlation (p<0.05-0.01) between serum leptin levels and both body weight and BMI in both groups, while IL-6 showed significant positive correlation (p<0.05-0.01) with BMI only at the start of the study.

Table 6 showed significant positive correlation at p<0.01 between IgA and both IgG and IgM in the all visits among group I, while significant correlation was detected between IgA and IgG at the first visit and IgM at the final visit in group 2 (Table 6).

Table 6: Correlation coefficient between the different serum immunoglobulins among the obese women at the different periods of intervention

	IgA					
	Group 1 (n = 22)			Group 2 (n = 8)		
Immunoglobulins	Base	Mid	Final	Base	Mid	Final
IgG	1.000**	0.861**	0.872**	1.000**	NS	NS
IgM	1.000**	0.864**	0.872**	NS	NS	0.983**

*: p<0.05, **: p<0.01

Table 7: The percent distribution of the obese women (group 1) according to immunoglobulin levels before, during and after intervention

	Deficient		Normal		Higher		Total		Standard range
Visits	No	%	No	%	No	%	No	%	(as the kit) (mg L^{-1})
IgA									
Base	--	--	3	14.29	18	85.71	21	100	490-2910
Mid	--	--	4	19.05	17	80.95	21	100	
Final	--	--	6	35.29	11	58.82	17	100	
IgG									
Base	6	28.57	3	14.29	12	57.14	21	100	6290-12650
Mid	3	14.29	4	19.05	14	66.67	21	100	
Final	1	5.88	6	35.29	10	58.82	17	100	
IgM									
Base	--	--	3	14.29	18	85.71	21	100	350-1800
Mid	--	--	4	19.05	17	80.95	21	100	
Final	--	--	7	41.18	10	64.71	17	100	

28.57% of the women were complain of IgG deficiency at the start of the study. The percent decrease reached 14.29 and 5.88% after dieting and supplementation. No such observation could be found among group 2 (Table 7).

DISCUSSION

Data from this study indicate that obesity is related to the high levels of the adipokine hormone leptin and the cytokine IL-6, while the serum concentration of the immunoglobulins IgA, IgG, IgM were at their low normal levels. It has been stated that obesity is associated with a chronic inflammatory response characterized by abnormal cytokine production, increase acute phase reactants and activation of inflammatory signaling pathways (Wellen and Hotamiligil, 2003). Critical questions include the mechanisms by which the inflammatory response is triggered and maintained in obesity. Previous studies illustrate that macrophage infiltration into adipose tissue could be integral to their inflammatory change (Xu *et al*., 2003; Weisberg *et al*., 2003). The stimulus for macrophages infiltration of adipose tissue may attributed to many causes, one of them, when adipocytes begin to secrete low level of tumor necrosis factor-α(TNF-α) which can stimulate preadipocytes to produce monocyte chemoattractant protein-1(MCP-1) which could be responsible for attracting macrophages to adipose tissue (Xu *et al*., 2003). Also the increased secretion of leptin hormone by adipocytes may be also the second cause contribute to macrophage accumulation by stimulating transport of macrophages to adipose tissue (Sierra-Honigmann *et al*., 1998).

After dieting most of the anthropometric parameters and the levels of the serum lipids among our obese women decreased significantly in both groups. The serum minerals concentration and the haemoglobin level either increase or decrease but all were within the normal ranges in both groups, this may pointed to the nutritional adequacy of the hypocaloric diet they were advised to follow, beside the vegetables soup consumed by group I.

The results of this study demonstrated that all the obese women were complained from hyperleptinaemia which showed significant correlation with body weight and BMI. Weight loss reduced the circulating leptin levels significantly, which concomitantly lower the serum level of the inflammatory marker IL-6 in group I by -14.90 and -17.86% in the mid and last periods of intervention. As mention before it has been hypothesized that elevated leptin levels underline the low grade proinflammatory state associated with human obesity (Loffreda *et al*., 1998). However the role of leptin on the IL-6 concentration was not quite clear in the obese women of the second group. It is interesting to mention here that the percent decrease in the waist circumference was less among the subjects of the second group, this may attributed to the persistant high level of the IL-6. It has been reported that obesity particular visceral adiposity is associated with chronic low-grade inflammation, as indicated by increased levels of the inflammatory marker C-reactive protein and IL-6 (Fantuzzi, 2005). In addition this group deprived the beneficial antioxidant and anti-inflammatory effect of the vegetables soup consumed by the first group. It has been reported that increased consumption of the fruits and vegetables containing high levels of phytochemicals has been recommended to prevent chronic diseases related to oxidative stress in human body (Liorach *et al*., 2004). Most of the antioxidant activity of the vegetables may come from their phenolic and flavonoids contents (Liu, 2003). Plant flavonoids show ant-inflammatory activity *in vitro* and *in vivo* (Kim *et al*., 2004). Recent studies have also shown that certain flavonoids express in part by modulating of proinflammatory gene expression such as cyclooxygenase 2 inducible nitric oxide synthase and several pivotal cytokines (Kim *et al*., 2004).

Evidence from *in vitro* and animal studies suggested that leptin is also involved in regulation of humoral inflammatory response through its direct effect on T cell (Bouloumie *et al*., 1999) monocytes (Fantuzz and Faggioni, 2000; Zarkesh-Esfahani *et al*., 2001), neutrophils (Caldefie-chezet *et al*., 2001) and endothelial cells (Bouloumie *et al*., 1999; Yamagishi *et al*., 2001). However inspite of the high level of leptin in obese subjects, yet serum levels of the immunoglobulins IgA, IgM and IgG were within the low normal range, in addition 28.5% of the obese women in group 1 were complained from IgG deficiency. It is possible to hypothesize that elevated leptin level in obese subjects can cause down modulation and/or desensitization of leptin receptors. This effect is perceived in the hypothalamus (centrally) and in the prephary as a condition of leptin deficiency, thus causing alteration of the immune response (Matarese and La Cuva, 2005). In this context clinical epidemiologic data indicate that the incidence and severity of specific types of infectious illnesses are higher in obese persons compared with lean person (Stallone, 1994; Moulton *et al*., 1996; Fasol *et al*., 1992; Weber *et al*., 1985). Data of this study showed that after dieting and the decrease in body weight and serum leptin and the improvement of its relative resistance, the levels of the three immunoglobulins improved significantly to reach their high normal range in both obese groups, thereby the percent of cases who were found to have IgG deficiency in the first group decrease to 14.29% after dieting for two weeks and to 5.88% at the end of the study. All the three immunoglobulin antibodies showed good response especially IgA and IgG in both groups, so they significantly correlated positively with each other especially among obese women in the first group. Immunoglobulins have many classes, each has a particular biological activity and acts primarily at different sites in the body (Samartin and Chandra, 2001). It is interested to say that the percent increase in the three immunoglobulin was also higher among obese women who were consumed vegetables soup compared to the control group. This could attributed to the flavonoids present in the vegetables as part of the polyphenols content, flavonoids have several biological activities including immunomodulating activities (Ielpo *et al*., 2000).

Thus it can be considered that obesity corresponds to a sub-clinical inflammatory condition with an increase level of IL-6, leptin and in the same time immune variations. The uses of vegetables soup rich in the phenolic compounds as a supplement with the low caloric balanced diet in the obese subjects proved to improve these effects.

REFERENCES

Abe, A., S. Yamashita and A. Nama, 1989. Sensitive, direct colorimetric assay for copper in serum. Clin. Chem., 35: 552-554.

Ahima, R.S. and J.S. Flier, 2000. Leptin. Anu. Rev. Physiol., 62: 413-437.

Anonymous, 2004. Global strategy on diet, physical activity and health. Fifty-Seven World Health Assembly.

AOAC (Association of Official Analysis Chemists), 1990. Official Methods of Analysis. 15th Edn. Washington DC: Association Official Analytical Chemists, 70: 668-931, 937.

Asatoor, A.M. and E.J. King, 1980. Blood Glucose and its Determination in Practical Clinical Biochemistry, V.I., Varley, H., A.H. Gowenlode and M. Bell (Eds.). 5th Edn., William Henemann Medical Books Ltd., London, pp: 397-399.

Bouloumie, A., T. Marumo, M. Lafontan and R. Busse, 1999. Leptin induces oxidative stress in human endothelial cells. FASEB., 13: 1231-1238.

Caldefie-chezet, F., A. Poulin, A. Tridon, B. Sion and M.P. Vasson, 2001. Leptin: A potential regulator of polymorphonuclear neutrophil bactericidal action? J. Leukocyte Biol., 69: 414-418.

Chase, G.W., W.O. Landen and G.M. Soliman, 1993. Method's modification for liquid chromatographic determination of thiamine, riboflavin and pyridoxine in medical foods. J. AOAC Inter., 76: 1267-1280.

Desia, I.D. and L.J. Machlin, 1985. Vitamin E in Methods of vitamin Assay, Augustin, J., B.P. Klein, D. Beker and P.B. Venugopal (Eds.). 4th Edn. A Wiley-Interscience Publication John Wiley and Sons, New York, pp: 255.

Eckel, R.H., W.W. Barouch and A.G. Ershow, 2002. Working group on the pathology of obesity-associated cardiovascular disease. Report of the National Heart, Lung and Blood Institute-national Institute of Diabetes and Digestive and Kidney Diseases. Circulation, 105: 2923-2928.

Elin, R.J., 1987. Assessment of magnesium status. Clin. Chem., 33: 1965-1970.

Faggoni, R., K.R. Feingold and C. Grunfeld, 2001. Leptin regulation of the immune response and the immunodeficiency of malnutrition. FASEB., 15: 2565-2571.

Fantuzz, G. and R. Faggioni, 2000. Leptin in the regulation of immunity, inflammation and haematopoieses. J. Leuukocyte Biol., 68: 437-446.

Fantuzzi, G., 2005. Adipose tissue, Adipokines and inflammation. J. Allergy Clin. Immunol., 115: 911-919.

Fasol, R., M. Schindler, B. Schumacher, K. Schlaudraff, W. Hannes, R. Seitelberger and V. Schlosser, 1992. The influence of obesity on perioperative morbidity: Retrospective study of 502 aortocoronary bypass operations. Thorac Cardiovasc Surg., 40: 126-129.

Guillaume, M. and P. Bjorntorp, 1996. Obesity in children, environmental and genetic aspects. Hoem. Metab. Res., 28: 573-581.

Homsher, R. and B. Zak, 1985. Determination of zinc plasma. Clin. Chem., 31: 1310-1313.

Hussein, L. and J. Bruggeman, 1997. Zinc analysis of Egyptian food and estimated daily intakes among an urban population group. Food Chem., 58: 391-398.

Ielpo, M.T.L., A. Basile, R. Miranda, V. Moscatiello, C. Nappo, S. Sorbo, E. Laghi, M.M. Ricciardi, L. Ricciardi, M.L. Vuotto, 2000. Immunopharma cological properties of flavonoids. Fitoterapia, 71: S101-S109.

Jelliffe, D.B., 1966. The assessment of the nutritional status of the community. World Health Organization. Geneva. Monograph., 35: 63-69.

Kim, H.P., K.H. Son, H.W. Chang and S.S. Kang, 2004. Anti-inflammatory plant flavonoids and cellular action mechanism. J. Pharmacol. Sci., 96: 229-245.

La Cava, A. and G. Matarese, 2004. The weight of leptin in immunity. Nat. Rev. Immunol., 4: 371-379.

Lavrovsky, Y., B. Chatterjee, R.A. Clark and A.K. Roy, 2000. Role of redox-regulated transcription factors in inflammation, aging and age-related diseases. Exp. Gerontol., 35: 521-531.

Liorach, R., F.A. Tomas-Barberan and F. Farreras, 2004. Lettuce and chicory byproducts as a source of antioxidant phenolic extracts. J. Agric. Food Chem., 52: 5109-5116.

Liu, R.H., 2003. Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. Am. J. Clin. Nut., 78 (3 Suppl): 517S-520S.

Loffreda, S., S.Q. Yang, H.Z. Lin, C.L. Karp, M.L. Brengman, D.J. Wang, A.S. Klein, G.B. Bulkley, C. Bao, P.W. Noble, M.D. Lane and A.M. Diehl, 1998. Leptin regulates proinflammatory immune responses. FASEB., 12: 57-65.

Macini, G., A.D. Carbonara and I.F. Heremans, 1965. Immuno-chemical quantification of antigens by single radial immunodiffusion. Immunochemistry, 2: 235-254.

Matarese, G. and A. La Cuva, 2005. Immune Responses in Obesity Models. Elsevier Ltd. All Rights Reserved. Drug.

Maxey, R.M., R.R. Maddipati and T. Birrmeier, 1992. Interference in immunoassay. J. Clin. Immunoassay, 15: 116-120.

Mohamed, A.V., S.A. Goodrick, D.R. Rawesh, J.M. Katz Miles, J.S. Yudkin, S. Klein and S.W. Coppack, 1997. Subcutaneous adipose tissue release interleukin-6 but not tumor necrosis factor-alpha, *in vitro*. J. Clin. Endocrinol. Metab., 82: 4196-4200.

Moulton, M.J., L.L. Creswell and M.E. Mackey *et al*., 1996. Obesity is not a risk factor for significant adverse outcome after cardiac surgery. Circulation, 94: 87-92.

Munoz, M., R.A. Mazure and J.M. Culebras, 2004. Obesity and the immune system. Nut. Hosp., 19: 319-324.

Samartin, S. and R.K. Chandra, 2001. Obesity, overnutrition and immune system. Nut. Rev., 21: 243-262.

Sierra-Honigmann, M.R., A.K. Nath, C. Murakami, G. Garcia-Cardena, A. Papapetropoulos, W.C. Sessa, L.A. Magda, J.S. Schechner, M.B. Schwabb, P.J. Polverini and J.R. Flores-Riveros, 1998. Biological action of leptin as an angiogenic factor. Science, 281: 1683-1686.

Simpson, K.L., C.S. Tsou and C.O. Chichester, 1985. Carotenes. In: Methods of Vitamin Assay, Augustin, J., B.P. Klein, D. Becker and P.B. Vengopal (Eds.). 4th Edn. A Widely Interscience Publication John Wiley and Sons, New York, pp: 85-202.

Singleton, V.L. and J.A. Rossi, 1965. Colorimetry of total phenolics with phosphomolybdic-Phosphotungstic acid reagents. Am. J. Enol. Vitic., 16: 144-158.

Stallone, D.D., 1994. The influence of obesity and its treatment on the immune system. Nut. Res., 52: 37-50.

Stein, E.A., 1986. Textbook of Clinical Chemistry, Tietz, N.W. (Ed.). W.B. Saunders, Philadelphia, pp: 879-886, 1818, 1829.

Tanaka, S., F. Isoda, T. Yamakawa, M. Ishihara and H. Sekihara, 1998. Tlymphopenia in genetically obese rats. Clin. Immunol. Immunopathol., 32: 113-120.

Van Kampen, E.J. and W.G. Zijlstra, 1961. Standardization of haemoglobinometry, the haemoglobincyanide method. Clin. Chim. Acta, 6: 538-543.

Wahlefeld, A.W., 1974. Methods of Enzymatic Analysis, Bergmeyer, H.U. (Ed.). Academic Press, New York, 5: 1831-1835.

Weber, D.J., W.A. Rutala and G.P. Samsa, 1985. Obesity as a predictor of poor antibody response to hepatitis B plasma vaccine. JAMA., 254: 3187-3189.

Weisberg, S.P., D. McCann, M. Desai, M. Rosenbaum, R.L. Leibe and A.W. Jr. Ferrante, 2003. Obesity is associated with macrophage accumulation in adipose tissue. J. Clin. Inves., 112:1796-1808.

Wellen, K.E. and G.S. Hotamiligil, 2003. Obesity-induced inflammatory changes in adipose tissue. J. Clin. Invest., 112: 1785-1788.

Xu, H., G.T. Barnes, Q. Yang, G. Tan, D. Yang, C.J. Chou, J. Sole, A. Nichols, J.S. Ross, L.A. Tartaglia and H. Chen, 2003. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. J. Clin. Invest., 112: 1821-1830.

Yamagishi, S.I., D. Edelsten, X.L. Du, Y. Kaneda, M. Guzman and M. Brownlee, 2001. Leptin induces mitochondrial superoxide production and monocyte chemoattractant protein-1 expression in aortic endothelial cells by increasing fatty acid oxidation via protein kinase A. J. Biol. Chem., 276: 25096-25100.

Zarkesh-Esfahani, H., G. Packley, R.A. Metcalf, M. Bidlingmaier, Z. Wu, A. Ajami, A.P. Weetman, C.J. Strasburger and R.J. Ross, 2001. High-dose leptin activates human leukocyte via receptor expression on monocytes. J. Immunol., 167: 4593-4599.