ORIGINAL ARTICLE
Iran J Allergy Asthma Immunol
June 2007; 6(2): 79-87

The Opposite Associations of Lycopene and Body Fat Mass with Humoral Immunity in Type 2 Diabetes Mellitus: A Possible Role in Atherogenesis

Tirang R. Neyestani, Nastaran Shariat-Zadeh, A'azam Gharavi, Ali Kalayi, and Niloufar Khalaji

Laboratory of Nutrition Research, National Nutrition and Food Technology Research Institute, Shaheed Beheshti University of Medical Sciences, Tehran, Iran

Received: 13 December 2006; Received in revised form: 28 December 2006; Accepted: 6 January 2007

ABSTRACT

This study examined the possible effects of lycopene at physiological dosage and body fat mass on the humoral immune response in patients with type 2 diabetes mellitus (T2DM).

A total of 35 patients with Typ2 diabetes mellitus from both sexes aged 54±9 yrs from the Iranian Diabetes Society were introduced into a double blind placebo controlled clinical trial conducted for 2 months. After a 2-week lycopene free diet washout period, patients were allocated to either lycopene supplementation group (10mg/d) (n=16) or placebo age- and sex matched group (n=19) for 8 weeks. Patients were instructed to keep their diets and physical activities as unchanged as possible.

Lycopene supplements increased serum lycopene levels ($p<0.001$). While intake of dietary energy and nutrients did not change in either groups, the ratio of total antioxidant capacity to malondialdehyde increased significantly in the lycopene group ($p=0.007$). There was an inverse correlation between serum levels of lycopene and those of IgG ($r= -0.338$, $p=0.008$). On the contrary, changes of serum levels of lycopene directly correlated with those of IgM ($r=0.466$, $p=0.005$). Interestingly, changes of the amount of fat mass correlated directly with those of serum IgG ($r=0.415$, $p=0.044$) but inversely with of serum IgM ($r= -0.469$, $p=0.021$).

While truncal fat might promote adaptive humoral immunity, lycopene probably by inhibiting MDA-LDL formation might attenuate T cell dependent adaptive (pro-atherogenic) humoral immune response. These findings may have preventive implications in long term diabetic complications, notably atherogenesis.

Key words: Diabetes mellitus Type 2; Fat mass; Immunity; Lycopene; Oxidative stress

INTRODUCTION

Type 2 diabetes mellitus (T2DM) usually threatens the affected individuals by its both short-term and long-term complications. While early diabetic complications are usually controlled by both nutritional and medication approaches, late complications, often more insidious and serious, may put the patients at risk for life-long.[1,2] Cardiovascular disorders (CVD) and atherosclerosis are among the most common long term diabetic complications.[3,4] Huge body of evidence proposes a role for oxidative stress, an imbalance in body proxidants and antioxidants towards proxidants, in initiation[5,6] as well as development[7,8] of

Corresponding Author: Tirang R. Neyestani, PhD;
Laboratory of Nutrition Research, National Nutrition and Food Technology Research Institute, Shaheed Beheshti University of Medical Sciences, P.O. Box 19395-4741, Tehran 1981619573, Iran.
Tel: (+98 21) 2235 7484-5 (ext. 288), Fax (+98 21) 2236 0660,
E-mail: tneyestani@nnftri.ac.ir

atherogenesis. Many experimental[9-11] and human [12-16] studies have examined the effects of different doses of various antioxidants in diabetes to attenuate oxidative stress, which is known to be augmented in diabetes.[17, 18] The results, however, have not been consistent as some studies failed to show the beneficial effects of antioxidant supplementation.[9, 13, 16]

Lycopene, the natural color of tomato and tomato products, is a potent antioxidant.[19] Meanwhile, the immune modulating effects of lycopene have been evaluated in many experimental [20] as well as human [21-23] studies. To investigate the *in vivo* antioxidant properties as well as immune modulating effects of lycopene at physiological dosage, we conducted a clinical trial on type 2 diabetic subjects. We found out, for the first time, that the amount of fat mass may also have a pivotal role on humoral immune response in T2DM.

PATIENTS AND METHODS

Study Design

Briefly, a placebo-controlled double blind clinical trial was conducted for 8 weeks on 35 patients with type 2 diabetes mellitus (T2DM) from the Iranian Society of Diabetes (ISD). A general overview was given to all patients attending ISD in a common session followed by a through explanation of the aim the study to the volunteers in a face to face interview. Then after, a written informed consent was taken. Patients were instructed to follow a lycopene-free diet for a two-week washout period. Anthropometric, dietary, biochemical and immunological assessments were done for all the patients both in the beginning (after washout) and after 8 week interventional period. This study was approved by the Ethical Committee of Shaheed Beheshti Medical University.

Subjects

A total of 35 patients with T2DM from both sexes (19 women and 16 men) aged 35-70 (54±9) years from ISD were introduced into the study. Patients who smoked, affected by other diseases like food allergy, auto immune disorders, liver, thyroid, and kidney diseases, and those who were taking vitamins C or E supplements or had to change their medications during interventional period were excluded. Duration of diabetes (since diagnosis) was less than 8 years. Subjects were allocated to either supplementation (n=16, 9 women and 7 men) or placebo (n=19, 10 women and 9 men) group and matched for age and sex. All patients were instructed to keep their diets and physical activities constant during the experimental period.

Anthropometry

Weight, height, waist and hip circumferences were measured and body mass index (BMI) was calculated using the equation $BMI=\frac{weight(kg)}{height(m)^2}$.

Dietary Assessment

To assess dietary intake, twenty four dietary recall for two days was used. Data were translated into energy and nutrients using Food Processor (FP) II software, which has been modified for Iranian foods.

Body Composition Analysis

Body composition was analyzed using bioelectrical impedance analysis (BIA) system (Quadscan 4000, BodyStat, UK) under fasting conditions. The system also estimated resting metabolic rate, which, in this study, was considered as basal metabolic rate (BMR).

Laboratory Investigations

Ten milliliter fasting venous blood sample was taken and divided in two tubes, either with or without anticoagulant. Sera from clot samples were recovered after 1 hour at room temperature (RT) followed by centrifugation at 2500g at RT for 20 minutes. Though fasting blood sugar (FBS) was measured immediately, the rest of the serum samples were transferred to -70°C in aliquots awaiting for further analysis. Saliva samples collected in fresh, clean capped glass bottles were also kept at -70°C to assess sIgA. Anticoagulated blood samples were analyzed for HbA1c at most 2 hr after sampling. Fasting blood glucose was determined using commercial enzymatic kit (Pars Azmoon, Iran). Glycated hemoglobin (HbA1c) was measured spectrophotometrically after initial separation by chromatography (G.D.S.r.l., Milan, Italy).

In this study serum total antioxidant capacity (TAC) to serum malondialdehyde (MDA) ratio was used to assess antioxidant/proxidant balance. TAC was determined using 2, 2”-azinobis (3-ethylbenzothiazoline-6-sulfonate) (ABTS) as a reagent. Potassium persulfate converts ABTS to ABTS cation radical ($ABTS^{\circ+}$), which is green-blue with maximum

absorption at 734 nm. Adding antioxidant solution to this in a given time decreases color intensity depending on the antioxidant activity and concentration. Therefore, decolorization will be expressed as a percent of $ABTS^{\circ+}$ inhibition based on difference of the primary and secondary absorbance divided by primary absorbance multiplied by 100.[24] Inhibition percent was compared with the antioxidant activity of bovine serum albumin (BSA) as standard. Results were expressed as mmol/L of BSA.

Serum MDA was determined using thiobarbituric acid reactive substances (TBARS) method described originally[25] with minor modifications. Briefly, proteins were precipitated using 20% trichloroacetic acid (TCA). The precipitate was washed with 0.05 M sulfuric acid before reacting with thiobarbituric acid (TBA) at 90-100 °C for 30 min. At pH 2-3, one molecule of MDA binds to two TBA molecules to form a pink complex. Following extraction with n-butanol, absorbance was determined at 532 nm against the n-butanol blank. Serum concentration of MDA was determined using a standard curve.

Humoral immunity was evaluated by determination of serum IgG, IgM and C3 as well as salivary sIgA concentrations using single radial immunodiffusion (SRID) method (Biogen, Iran).

To detect possible subtle changes in humoral immune response, serum auto antibodies to ox-LDL (anti-ox-LDL Abs) was also determined using double sandwich enzyme linked immunosorbent assay (ELISA) technique (Biomedica, Biomedica Gruppe, Austria).

Statistical Analyses

Normality of data distribution was evaluated using Kolmogrov-Smirnov. Comparison of means was done with student *t* test or, when the distribution was not normal, Mann-Whitney U-Wilcoxon. Correlations were determined using Pearson equation. The predetermined upper limit of significance throughout this investigation was $p<0.05$. All statistical analyses were done with Windows/SPSS version 11.5 package.

RESULTS

With the exception of TAC/MDA and anti-ox-LDL IgG, almost all data had a normal distribution as evaluated by Kolmogrov-Smirnov. Energy and nutrient intakes did not show any significant difference between two groups both in the beginning and in the end of the interventional period (Table 1). Nor was there any significant difference in anthropometric and body composition data between lycopene and placebo groups (Table 2).

However, serum lycopene levels increased significantly only in the lycopene group ($p<0.001$) while serum concentrations of β-carotene did not show any statistically significant change in the both groups (Figure 1), indicating that lycopene supplement increased serum lycopene levels. While mean serum concentration of MDA showed about 0.6nM/mL decrease in lycopene group, there was about 0.4nM/mL increase in the placebo group.This difference was statistically significant ($p=0.003$). Lycopene supplementation did not cause any significant change in serum TAC levels. However, TAC/MDA ratio increased significantly in the lycopene group (Mann-Whitney U-Wilcoxon, $p=0.007$) (Figure 2).

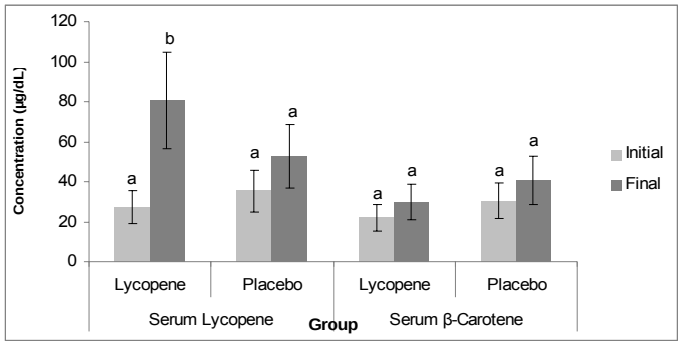

Figure 1. Initial and final serum levels of lycopene and β-carotene in the supplementation and placebo groups. Only in the supplementation group serum levels of lycopene increased significantly ($p<0.001$). Bars with different superscript letters indicate significant difference ($p<0.05$).

Table 1. Estimated daily energy and some selected nutrient intakes in lycopene supplementation (n=16) and placebo (n=19) groups.

Food Ingredient \ Group		Lycopene (n=16)	Placebo (n=19)
Energy (Kcal)	Initial	1395.3±630.6	1489.9±746.2
	Final	1410.8±682.7	1635.8±439.8
	Difference	0.9±12.5	2.5±14.8
Protein (g)	Initial	40.4±18.3	56.5±21.6
	Final	56.2±24.9	68.8±23.9
	Difference	18.4±18.3	12.2±32.3
Carbohydrate (g)	Initial	165.6±92.9	166.7±106.1
	Final	167.6±85.8	199.1±70.4
	Difference	19.9±53.2	32.4±117.7
Total Fat (g)	Initial	66.8±31.7	69.9±40.9
	Final	60.1±38.4	66.5±28.3
	Difference	-5.0±20.2	-3.4±23.9
Fiber (g)	Initial	16.1±8.4	19.0±13.3
	Final	16.2±8.0	21.5±13.2
	Difference	-0.9±12.5	-2.5±14.8
Vitamin C (mg)	Initial	107.8±87.9	108.8±106.4
	Final	77.2±87.2	84.7±81.9
	Difference	-34.7±127.1	-24.1±102.1
Vitamin E (mg)	Initial	27.6±18.9	28.7±19.8
	Final	26.4±19.6	27.3±18.8
	Difference	-2.7±8.6	-1.3±9.4
Selenium (mg)	Initial	107.8±87.9	108.8±106.4
	Final	77.2±87.2	84.7±81.9
	Difference	19.9±36.4	46.8±74.3
Zinc (mg)	Initial	5.6±3.1	7.6±2.7
	Final	6.7±2.6	9.6±4.6
	Difference	2.0±2.0	2.0±4.9

Values are mean ± SD.

Serum levels of IgG and salivary sIgA did not change significantly. Mean IgM levels in the lycopene group showed 15mg/dL increment while in the placebo group there was a mild decrease in serum IgM (6mg/dL). These changes were, however, not significant (Table 3). Serum levels of anti-ox-LDL IgG also showed some interesting, but insignificant, changes as there was a decrease in the lycopene group (-16.2 mIU/mL) but a small increase in the control group (+8.5 mIU/mL) (Figure 3).

Significant negative correlations between changes of serum levels of lycopene and MDA ($r=-0.56$, $p<0.001$) and between serum levels of lycopene and IgG ($r=-0.338$, $p=0.008$) were found, while serum levels of lycopene and IgM correlated positively ($r=0.466$, $p=0.005$). Changes of serum MDA and FBS showed similar correlation ($r=0.466$, $p=0.005$).

There was a positive correlation between serum concentrations of glucose and those of anti-ox-LDL IgG ($r=0.483$, $p=0.012$).

Table 2. Some selected anthropometric data of lycopene supplementation and placebo groups.

Characteristic \ Group		Lycopene (n=16)	Placebo (n=19)
Age		56.0±8.5	54.9±8.5
Weight (Kg)	Initial	70.8±12.5	75.3±10.9
	Final	68.7±9.7	75.4±10.5
	Diffference	-2.1±3.9	0.1±1.6
BMI (Kg/m^2)	Initial	27.1±3.7	28.8±4
	Final	26.1±2.5	28.8±4.3
	Diffference	-0.9±1.6	0.02±0.6
Waist (cm)	Initial	101.0±7.5	102.5±8.5
	Final	98.6±6.9	103.9±9.3
	Diffference	-2.4±6.9	1.4±5.6
WHR	Initial	0.98±0.04	0.95±0.06
	Final	0.95±0.07	0.95±0.05
	Diffference	-0.03±0.07	-0.003±0.04
BMR (Kcal)	Initial	1446.8±211.7	1576.0±267.9
	Final	1454.1±226.9	1551.1±265.4
	Diffference	7.4±36.3	-24.9±91.5
FM (% of B.W.)	Initial	22.7±7.6	25.4±8.0
	Final	21.2±6.8	24.9±8.2
	Diffference	-1.5±2.3	0.2±1.5

Abbreviations: BMI: body mass index; B.W.: body weight; BMR: basal metabolic rate; FM: fat mass; WHR: waist to hip ratio.

As a whole, body weight and increased (truncal) fat reserves showed direct correlations with serum IgG and MDA levels but inverse correlations with serum IgM and C3 levels (table 4).

For instance, changes of the amount of fat mass (FM) correlated directly with those of serum IgG (r=0.415, p=0.044) but inversely with of serum IgM (r= -0.469, p=0.021).

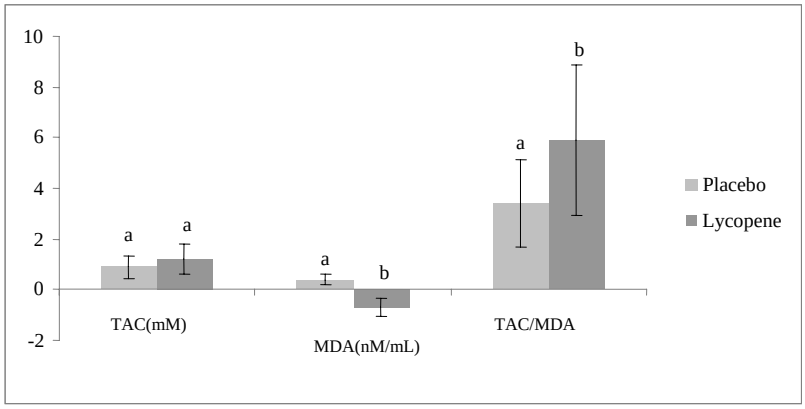

Figure 2. Changes of oxidative stress status of both lycopene supplemented and placebo control diabetic groups. A total of 35 type 2 diabetic patients were given either a daily dose of 10mg lycopene (n=16) or placebo (n=19) for 8 weeks. In the lycopene group, serum MDA was decreased while TAC/MDA was increased significantly indicating attenuation of oxidative stress. Bars represent mean values.

* Values (mean±SD) with different superscripts are significantly different (p< 0.05). The mean values of TAC/MDA were compared using Mann-Whitney U Wilcoxon.

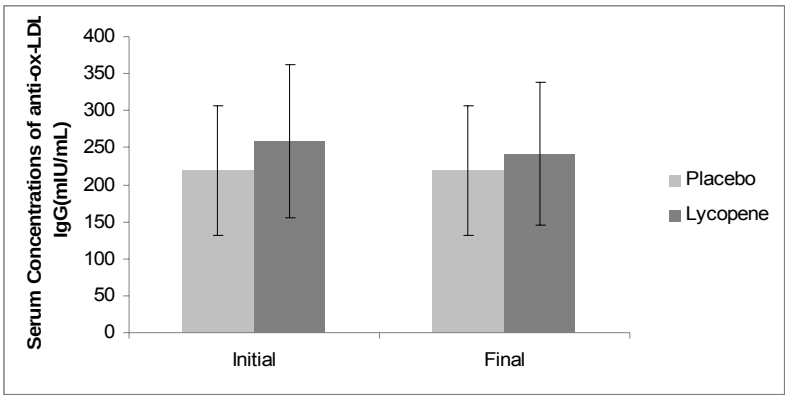

Figure 3. Serum concentration changes of anti-oxidized low-density lipoprotein (anti-ox-LDL) IgG after eight weeks treatment with either 10 mg/d lycopene (n=16) or placebo (n=19) in type 2 diabetic patients. Though serum levels of anti-ox-LDL IgG decreased in lycopene group and increased in placebo group, the changes were not statistically significant. Bars represent mean values±SD.

Table 3. Changes of some selected analytes in lycopene supplementation (n=16) and placebo (n=19) groups.

Analyte \ Group		Lycopene	Placebo
HbA1c (%)	Initial	10.0±1.7	9.5±1.8
	Final	9.3±1.3	8.7±1.4
	Difference	-0.7±1.7	-0.8±2.4
C3 (mg/dL)	Initial	103.2±28.4	85.7±27.0
	Final	111.0±41.7	99.3±32.8
	Difference	7.8±40.2	13.6±48.7
sIgA (mg/dL)	Initial	9.3±3.8	9.4±2.6
	Final	10±3.4	17.4±32.9
	Difference	0.7±2.3	7.9±32.4
IgM (mg/dL)	Initial	207.7±35.7	219.4±71.2
	Final	227.2±42.6	215.4±57.7
	Difference	19.5±36.9	-5.9±47.5
IgG (mg/dL)	Initial	1471.3±572.8	1422.3±318.6
	Final	1462.8±572.9	1251.8±261.7
	Difference	-8.4±579.5	-170.5±377.4

Values are mean ± SD. Different superscript letters in a row for each analyte denote significant difference ($p<0.05$).

Table 4. Some selected correlations between variables under study.

Variable	Weight (Kg)	BMI (Kg/m^2)	Waist Circumference (cm)	WHR	FM (%)
IgG (mg/dL)	r = 0.494	r = 0.495	r = 0.400	---	r = 0.415
	p = 0.003	p =0.003	p = 0.017		p = 0.044
IgM (mg/dL)	---	---	---	---	r = -0.469
					p = 0.021
C3 (mg/dL)	r = -0.383	---	r = -0.357	---	---
	p = 0.023		p = 0.035		
MDA (nM/mL)	---	r = 0.386	r = 0.353		
		p = 0.022	p = 0.038		

BMI: body mass index; FM: fat mass; MDA: malondialdehyde; WHR: waist to hip ratio

DISCUSSION

Immune cell functions, including signal transduction and gene expression, are generally believed to be influenced by the oxidant/antioxidant balance.[26] Antioxidants are therefore expected to have, at least theoretically, modulating effects on the immune response.

Atherosclerosis is known as an inflammatory disease, in which both innate and adaptive arms of immunity are involved. [27-30] On the other hand, the role of oxidative stress as an independent factor in pathogenesis of CVD should not be neglected. [17, 31] Atherosclerotic lesion formation may be promoted by LDL modifications, including oxidation, via different mechanisms.[32] The products of minimally modified (oxidized) LDL (mm-LDL) and fully oxidized LDL (ox-LDL), for instance, may directly or indirectly cause T cell and monocyte recruitment and differentiation of monocyte to macrophage.[33] These structural modifications of LDL may cause neo-self determinants formation. [32] Under oxidative stress conditions and *in vivo*, LDL molecule may be modified associating with fragmentation of polyunsaturated fatty acids and MDA formation. The conformational changes in the lipoprotein molecule may be enough to produce foreign-like structures and induce specific T cell immune response and specific antibody formation. These antibodies, usually pro-atherogenic and highly associated with the development of lesions, are mostly of IgG isotype.[32] In fact, IgG response, mostly related to adaptive immunity, may enhance macrophage uptake of ox-LDL-IgG through Fc-associated mechanisms. On the contrary, natural antibodies (usually of IgM and IgA isotypes), mostly related to innate immunity, may bind to and inhibit macrophage uptake of ox-LDL particles and hence promote plasma clearance of LDL containing oxidized phospholipids epitopes.[34]

Our interesting new finding on positive correlation of serum lycopene and IgM levels may be very important as it indicates that lycopene is not just a common immune enhancer, but it is probably associated with the innate arm of the immune response (anti-atherogenic) while modulates the other aspects of the immunity including specific T cell-dependent (pro-atherogenic) response.

Increased serum lycopene levels followed by attenuated oxidative stress status in the lycopene group indicated that lycopene, by inhibition of atherogenic MDA-LDL formation, may have an inhibitory potential in macrophage uptake of ox-LDL, foam cell formation and stimulation of pro-atherogenic specific T cell-dependent response. Accordingly serum levels of anti-ox-LDL Abs tended to be lower in the lycopene group. This hypothesis is further supported by the finding that in subjects with high concentrations of lycopene in their adipose tissue, there is reduced thickness of the endothelium lining blood vessels and hence risk of heart attack.[35]

Direct correlations of serum IgG with weight, BMI, waist circumference and, above all, FM on one hand and inverse correlations of serum C3 with waist circumference and IgM with FM, on the other, all well indicate that abdominal obesity simultaneously enhances adaptive arm (atherogenic) and attenuates innate arm (anti-atherogenic) of humoral immunity. These changes may have some role in atherogenesis.

Augmented oxidative stress, independent of blood glucose level fluctuations, has been documented in obesity (especially in abdominal type).[36] As T2DM is usually accompanied by central obesity, [37, 38] and chronic hyperglycemia *per se* induces oxidative stress, [18, 39, 40] diabetic patients need especial dietary care both for weight control and antioxidants. Many studies have used different antioxidants to suppress oxidative stress in T2DM, [9-12, 14-16] some of which failed to show the beneficial effect of the antioxidant at the dosages used in their investigation.[9, 16]

More important than the effect of lycopene intake on serum levels of lycopene, which is supportive of some other reports, [40] our findings showed that lycopene even at physiological dose could suppress lipid peroxidation in a well-known augmented oxidative stress situation. The direct relationship between serum levels of glucose and anti-ox-LDL Abs, on one hand, and between changes of serum glucose and MDA, on the other hand, both confirm our previous findings on the effect of serum glucose optimization on reduction of LDL oxidation [41] and hence neo-self antigen formation.

To the best of our knowledge, this is the first report on the opposite associations of lycopene and fat mass with humoral immunity in T2DM. Based on our findings, we propose that lycopene, as a potent antioxidant, inhibits lipid peroxidation and formation of MDA and MDA-LDL with neo-epitopes that stimulate

pro-atherogenic specific T cell-dependent response and specific antibody formation, mostly of IgG isotype. Meanwhile, lycopene by enhancing production of natural antibodies (especially of the IgM isotype), which are mostly related to innate immunity, may interfere with macrophage uptake of ox-LDL followed by foam cell formation. Increased fat reserves especially in the truncal area, often found in type 2 diabetic patients, may in part enhance T cell-dependent adaptive immune response probably through inflammatory adipocytokines.

ACKNOWLEDGEMENTS

This research project was funded by the National Nutrition and Food Technology Research Institute. All laboratory bench-works were done in the Laboratory of Nutrition Research, National Nutrition and Food Technology Research Institute. We do thank Dr. Asadollah Rajab, the Head of the Iranian Diabetes Society, for his sincere co-operation and all the patients who took part in this project just with the hope to find out some ways to make life easier for diabetics.

REFERENCES

1. Turner RC. The U.K. Prospective Diabetes Study. A review. Diabetes Care 1998; 21 Suppl 3:C35-8.
2. Nicolucci A, Carinci F, Ciampi A. Stratifying patients at risk of diabetic complications: an integrated look at clinical, socioeconomic, and care-related factors. SID-AMD Italian Study Group for the Implementation of the St. Vincent Declaration. Diabetes Care 1998; 21(9):1439-44.
3. Bo S, Ciccone G, Gancia R, Rosato R, Grassi G, Merletti F, et al. Mortality within the first 10 years of the disease in type 2 diabetic patients. Nutr Metab Cardiovasc Dis 2006; 16(1):8-12.
4. Hatunic M, Burns N, Finucane F, Mannion C, Nolan JJ. Contrasting clinical and cardiovascular risk status between early and later onset type 2 diabetes. Diab Vasc Dis Res 2005; 2(2):73-5.
5. Chen X, Niroomand F, Liu Z, Zankl A, Katus HA, Jahn L, et al. Expression of nitric oxide related enzymes in coronary heart disease. Basic Res Cardiol 2006; 101(4):346-53.
6. Madamanchi NR, Tchivilev I, Runge M. Genetic markers of oxidative stress and coronary atherosclerosis. Curr Atheroscler Rep 2006; 8(3):177-83.
7. Ueda S, Yasunari K. What we learnt from randomized clinical trials and cohort studies of antioxidant vitamin? Focus on vitamin E and cardiovascular disease Curr Pharm Biotechnol 2006; 7(2):69-72.
8. Minuz P, Fava C, Cominacini L. Oxidative stress, antioxidants, and vascular damage. Br J Clin Pharmacol 2006; 61(6):774-7.
9. Xia Z, Guo Z, Nagareddy PR, Yuen V, Yeung E, McNeill JH. Antioxidant N-acetylcysteine restores myocardial Mn-SOD activity and attenuates myocardial dysfunction in diabetic rats. Eur J Pharmacol 2006; 544(1-3):118-25.
10. Song MK, Rosenthal MJ, Song AM, Yang H, Ao Y, Yamaguchi DT. Raw vegetable food containing high cyclo (his-pro) improved insulin sensitivity and body weight control.Metabolism 2005; 54(11):1480-9.
11. Gur S, Karahan ST, Ozturk B, Badilli M. Effect of ascorbic acid treatment on endothelium-dependent and neurogenic relaxation of corpus cavernosum from middle-aged non-insulin dependent diabetic rats. Int J Urol 2005; 12(9):821-8.
12. Anderson RA, Evans LM, Ellis GR, Khan N, Morris K, Jackson SK, et al. Prolonged deterioration of endothelial dysfunction in response to postprandial lipaemia is attenuated by vitamin C in Type 2 diabetes. Diabet Med 2006; 23(3):258-64.
13. Clarke MW, Ward NC, Wu JH, Hodgson JM, Puddey IB, Croft KD. Supplementation with mixed tocopherols increases serum and blood cell gamma-tocopherol but does not alter biomarkers of platelet activation in subjects with type 2 diabetes. Am J Clin Nutr 2006; 83(1):95-102.
14. Neri S, Signorelli SS, Torrisi B, Pulvirenti D, Mauceri B, Abate G, et al. Effects of antioxidant supplementation on postprandial oxidative stress and endothelial dysfunction: a single-blind, 15-day clinical trial in patients with untreated type 2 diabetes, subjects with impaired glucose tolerance, and healthy controls. Clin Ther 2005; 27(11):1764-73.
15. Baliarsingh S, Beg ZH, Ahmad J. The therapeutic impacts of tocotrienols in type 2 diabetic patients with hyperlipidemia. Atherosclerosis 2005; 182(2):367-74.
16. Chen H, Karne RJ, Hall G, Campia U, Panza JA, Cannon RO 3rd, et al. High-dose oral vitamin C partially replenishes vitamin C levels in patients with Type 2 diabetes and low vitamin C levels but does not improve endothelial dysfunction or insulin resistance. Am J Physiol Heart Circ Physiol 2006; 290(1):H137-45.
17. Stephens JW, Gable DR, Hurel SJ, Miller GJ, Cooper JA, Humphries SE. Increased plasma markers of oxidative stress are associated with coronary heart disease in males with diabetes mellitus and with 10-year risk in a prospective sample of males. Clin Chem 2006; 52(3):446-52.
18. Lankin VZ, Lisina MO, Arzamastseva NE, Konovalova GG, Nedosugova LV, Kaminnyi AI, et al. Oxidative

stress in atherosclerosis and diabetes. Bull Exp Biol Med 2005; 140(1):41-3.
19. Weisburger JH. Lycopene and tomato products in health promotion. Exp Biol Med (Maywood) 2002; 227(10):924-7.
20. Kim GY, Kim JH, Ahn SC, Lee HJ, Moon DO, Lee CM, et al. Lycopene suppresses the lipopolysaccharide-induced phenotypic and functional maturation of murine dendritic cells through inhibition of mitogen-activated protein kinases and nuclear factor-kappaB. Immunology 2004; 113(2):203-11
21. Briviba K, Kulling SE, Moseneder J, Watzl B, Rechkemmer G, Bub A, et al. Effects of supplementing a low-carotenoid diet with a tomato extract for 2 weeks on endogenous levels of DNA single strand breaks and immune functions in healthy non-smokers and smokers. Carcinogenesis 2004; 25(12):2373-8.
22. Watzl B, Bub A, Briviba K, Rechkemmer G. Supplementation of a low-carotenoid diet with tomato or carrot juice modulates immune functions in healthy men. Ann Nutr Metab 2003; 47(6):255-61.
23. Watzl B, Bub A, Brandstetter BR, Rechkemmer G. Modulation of human T-lymphocyte functions by the consumption of carotenoid-rich vegetables. Br J Nutr 1999; 82(5):383-9.
24. Rice-Evans, Miller NJ. Total antioxidant status in plasma and body fluids. Methods Enzymol 1994; 234:279-93.
25. Satoh K. Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. Clin Chim Acta 1978; 90(1):37-43.
26. Meydani SN, Wu D, Santos MS, Hayek MG. Antioxidants and immune response in aged persons: overview of present evidence. Am J Clin Nutr 1995; 62(6 Suppl):1462S-1476S.
27. Gurfinkel E, Lernoud V. The role of infection and immunity in atherosclerosis. Expert Rev Cardiovasc Ther 2006; 4(1):131-7.
28. Kobayashi K, Lopez LR, Shoenfeld Y, Matsuura E. The role of innate and adaptive immunity to oxidized low-density lipoprotein in the development of atherosclerosis. Ann N Y Acad Sci 2005; 1051:442-54.
29. Nilsson J. Regulating protective immunity in atherosclerosis. Circ Res 2005; 96(4):395-7.
30. Caligiuri G, Nicoletti A, Poirier B, Hansson GK. Protective immunity against atherosclerosis carried by B cells of hypercholesterolemic mice. J Clin Invest 2002; 109(6):745-53.
31. Saha A, Adak S, Chowdhury S, Bhattacharyya M. Enhanced oxygen releasing capacity and oxidative stress in diabetes mellitus and diabetes mellitus-associated cardiovascular disease: a comparative study. Clin Chim Acta 2005; 361(1-2):141-9.
32. Shaw PX. Rethinking oxidized low-density lipoprotein, its role in atherogenesis and the immune responses associated with it. Arch Immunol Ther Exp (Warsz) 2004; 52(4):225-39.
33. Weber C, Erl W, Weber PC. Enhancement of monocyte adhesion to endothelial cells by oxidatively modified low-density lipoprotein is mediated by activation of CD11b. Biochem Biophys Res Commun 1995; 206(2):621-8.
34. Horkko S, Miller E, Dudl E, Reaven P, Curtiss LK, Zvaifler NJ, et al. Antiphospholipid antibodies are directed against epitopes of oxidized phospholipids. Recognition of cardiolipin by monoclonal antibodies to epitopes of oxidized low density lipoprotein. J Clin Invest 1996; 98(3):815-25.
35. Arab L, Steck S. Lycopene and cardiovascular disease. Am J Clin Nutr 2000; 71(Suppl):1691S-5S.
36. Furukawa S, Fujita T, Shimabukuro M, Iwaki M, Yamada Y, Nakajima Y, et al. Increased oxidative stress in obesity and its impact on metabolic syndrome. J Clin Invest 2004; 114(12):1752-61.
37. Sibley SD, Thomas W, de Boer I, Brunzell JD, Steffes MW. Gender and elevated albumin excretion in the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) cohort: role of central obesity. Am J Kidney Dis 2006; 47(2):223-32.
38 McTernan CL, McTernan PG, Harte AL, Levick PL, Barnett AH, Kumar S. Resistin, central obesity, and type 2 diabetes. Lancet 2002; 359(9300):46-7.
39. EI Midaoui AE, de Champlain J. Effects of glucose and insulin on the development of oxidative stress and hypertension in animal models of type 1 and type 2 diabetes. J Hypertens 2005; 23(3):581-8.
40. Ganji V, Kafai MR; Third National Health and Nutrition Examination Survey, 1998-1994. Population determinants of serum lycopene concentrations in the United States: data from the Third National Health and Nutrition Examination Survey, 1988-1994. J Nutr 2005; 135(3):567-72.
41. Neyestani TR, Alipour-Birgani R, Siassi F, Rajayi M, Djalali M, Mohamadi M. Glycemic optimization may reduce lipid peroxidation independent of weight and blood lipid changes in Type 2 diabetes mellitus. Diabetes Nutr Metab 2004; 17(5):275-9.