The Effect of Tomato-Derived Lycopene on Low Carotenoids and Enhanced Systemic Inflammation and Oxidation in Severe Obesity

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ABSTRACT: **Background:** Fat tissue mediates the production of inflammatory cytokines and oxidative products, which are key steps in the development of type 2 diabetes and atherosclerosis. Antioxidant-rich diets protect against chronic diseases. Antioxidants may interfere with pro-inflammatory signals.

Objectives: To investigate the effect of the potent tomato-derived antioxidant carotenoid, lycopene, on plasma antioxidants (carotenoids and vitamin E), inflammatory markers (C-reactive protein, interleukin-6, tumor necrosis factor-alpha) and oxidation products (conjugated dienes).

Methods: Eight obese patients (body mass index 37.5 ± 2.5 kg/m^2) were compared with a control group of eight lean, age and gender-matched subjects (BMI 21.6 ± 0.6 kg/m^2), before and after 4 weeks of lycopene supplementation (tomato-derived Lyc-O-Mato) (30 mg daily).

Results: Plasma carotenoids were significantly reduced in the obese compared to control subjects (0.54 ± 0.06 vs. 0.87 ± 0.08 µg/ml, $P < 0.01$). CRP levels were significantly higher (6.5 vs. 1.1 mg/L, $P = 0.04$) in obese vs. controls, as were IL-6 and conjugated dienes (3.6 and 7.9-fold, respectively). CRP, IL-6 and conjugated dienes correlated with BMI, while IL-6 and conjugated dienes correlated inversely with carotenoids ($P < 0.05$). Following lycopene treatment, a significant elevation of plasma carotenoids (1.79 vs. 0.54 µg/ml) and specifically lycopene (1.15 vs 0.23 µg/ml) ($P < 0.001$) occurred in the treatment vs. the placebo group, respectively. Markers of inflammation and oxidation products were not altered by lycopene.

Conclusions: Obese patients showed abnormally higher markers of inflammation and oxidation products and lower plasma carotenoids. The lack of reduction of pro-inflammatory markers could be attributed to the short period of the study and the small number of participants. More studies are needed on the protective qualities of natural antioxidant-rich diets against obesity-related co-morbidities.

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KEY WORDS: obesity, inflammation, C-reactive protein, carotenoids, lycopene

The prevalence of obesity (body mass index ≥ 30 kg/m^2) is steadily growing, with more than 50% in certain populations in Israel [1]. Obesity is strongly correlated with cardiovascular morbidity and mortality [2]. The adipose tissue secretes inflammatory cytokines such as interleukin-6 and tumor necrosis factor-alpha, which contribute to atherosclerosis by inducing insulin resistance and up-regulating the expression of other inflammatory mediators. IL-6 enhances liver production of C-reactive protein. Elevated levels of plasma CRP, IL-6 and TNFα, all found in obesity, are independent predictors of cardiovascular disease [3]. Moreover, TNFα and IL-6 are significant predictors of the extent of coronary artery disease [4]. CRP, which predicts acute coronary events, strokes and peripheral vascular disease, has been widely studied.

Cardiovascular morbidity and mortality may decrease by reducing the levels of chronic inflammation and oxidative stress. Carotenoids are well-known lipid-soluble antioxidants, sourcing mainly from fruit and vegetables. Carotenoids cause changes in the expression of many proteins participating in cell proliferation and signaling pathways. Diets rich in tomato products are associated with decreased risk of chronic diseases [5].

Lycopene, the predominant tomato carotenoid, is the most potent antioxidant among plasma carotenoids. However, its mode of action has been attributed to other mechanisms and much has been learned from its protection against prostate cancer. Lycopene accumulates in both normal and malignant prostate gland upon supplementation and reduces transcript levels of pro-inflammatory cytokines with down-regulation of IL-6 expression [6]. Lycopene increases in adipose tissue in humans [7] where it may exert its action on cytokine pathways. Mediterranean-like meals rich in carotenoids lead to a postprandial decrease in CRP [8]. Therefore, we planned to follow the effect of tomato-derived lycopene consumption on markers of inflammation and oxidative stress in a group of patients with extreme obesity.

BMI = body mass index
CRP = C-reactive protein
IL = interleukin
TNFα = tumor necrosis factor-alpha

PATIENTS AND METHODS

Eight subjects (47 ± 6 years, 4:4 male/female) with obesity (BMI 37.5 ± 2.5 kg/m^2) participated in the study. All subjects signed an informed consent approved by the Helsinki Committee of Rambam Medical Center. In addition, eight healthy, lean, age and gender-matched control subjects (BMI 21.6 ± 0.6 kg/m^2) were analyzed for plasma antioxidants and inflammatory markers after a 12 hour fast. This group was recruited from the annual checkup program of the hospital after exclusion of acute or chronic diseases (diabetes, hypertension, hyperlipidemia, etc.) that may have affected cytokine levels. This group was not supplemented with lycopene.

For a period of 8 weeks each patient was supplemented with two capsules daily. The first four weeks were a placebo run-in period, followed by 4 weeks of lycopene supplementation, 30 mg daily (Lyc-o-Mato®; LycoRed Natural Products Industries Ltd., Beer Sheva, Israel). Lyc-o-Mato is an enriched lycopene product derived from tomatoes that contain other bioactive ingredients, such as tocopherols, phytoene and phytofluene. The placebo capsules, made of edible soya oil, were carotenoid free.

Patients were instructed to keep a normal diet and activity. Patients consumed between one and three tomatoes (average two) with one to three vegetables and up to two fruit servings daily. Taking both placebo and lycopene, each obese subject behaved as a self-control, thus minimizing any confounding factors that may have had an undesired impact on the levels of plasma antioxidants and markers of inflammation due to the heterogenous nature of the group.

Blood was taken at three stages: at the beginning of the study, after 4 weeks of placebo and after 4 weeks on lycopene. Plasma was separated and kept at -20°C until analyzed. The following tests were performed: Metabolic profile (glucose and lipids) by a Hitachi Auto Analyzer, and high density lipoprotein-cholesterol (by phosphotungstate method). Insulin was analyzed using Microparticle Enzyme Immunoassay-Axsym Insulin Assay (Abbot). CRP was measured by a high sensitivity CRP kit (DADE Behring) using a particle-enhanced immunonephelometry method (with a lower detection limit of 0.15 mg/L) [9]. IL-6 and TNFα were analyzed by a Sandwich enzyme immunoassay. Total plasma carotenoids, vitamin E, lycopene and oxidation products (conjugated dienes) were determined by a high performance liquid chromatography analysis in the National Institute of Oceanography, as previously described [10]. The metabolic and inflammatory profiles were determined in the clinical biochemistry and endocrinology laboratories of Rambam Medical Center which adhere to strict quality control standards.

All measurements are presented as mean ± standard error (SEM). *t*-test was used to compare between the study group and the controls. Pearson's correlation coefficients between different variables were calculated. Tukey's post hoc test was used to assess the effect of lycopene treatment. *P* values less than 0.05 represent statistical significance. All calculations were done by a skilled biostatistician.

RESULTS

BMI (kg/m^2) remained constant throughout the study (37.5 ± 2.5, 37.6 ± 2.5, 37.5 ± 2.6, at the beginning of the study, after placebo and after lycopene, respectively).

Table 1 illustrates the metabolic, inflammatory and oxidative characteristics of the obese patients compared to the lean control subjects. The obese patients had normal-high fasting glucose and insulin levels, with dyslipidemia. Also, they had significantly higher plasma CRP, IL-6 and conjugated dienes (6, 3.6 and 7.9-fold, respectively). TNFα was not different between these two groups. Table 1 shows the concentrations of antioxidants in the obese patients at baseline (prior to supplementation) compared to lean controls. Plasma carotenoids were significantly (38%) lower among obese patients. Lycopene levels were higher among the obese but did not reach significance. Vitamin E was significantly higher in the obese patients.

MARKERS OF INFLAMMATION AND OXIDATION

CRP, IL-6 and conjugated dienes correlated significantly and increased with BMI [Table 2]. Whereas these significant correlations (between CRP, IL-6, conjugated dienes and BMI) persisted throughout the entire study (at start, after placebo, and after treatment), no correlations were observed between TNFα and BMI, at any phase. Plasma IL-6 and conjugated dienes correlated significantly and inversely with plasma carotenoids while CRP and TNFα did not [Table 2]. There

Table 1. Metabolic, inflammatory and oxidative markers in obese and lean subjects

	Obese (n=8)	Control (n=8)	*P*
BMI (kg/m^2)	37.5± 2.5	21.6 ± 0.6	‹ 0.001
Glucose (mg/dl)	107.4 ± 9.8	92.2 ± 2.6	NS
Insulin (pmol/L)	147.4 ± 27.1	ND	
LDL (mg/dl)	145.7 ± 15.3	118.5 ± 8.7	NS
HDL (mg/dl)	43.2 ± 2.8	53.3 ± 2.2	NS
Triglycerides (mg/dl)	199.4 ± 43.4	98.0 ± 15.1	‹ 0.01
CRP (mg/L)	6.5 ± 2.4	1.1 ± 0.3	0.044
IL-6 (pg/ml)	3.6 ± 0.4	1.0 ± 0.2	‹ 0.0001
TNF-α (pg/ml)	1.4 ± 0.2	1.4 ± 0.3	NS
Dienes (a.u)	181 ± 18	23 ± 2.0	‹ 0.0001
Carotenoids (μg/ml)	0.54 ± 0.06	0.87 ± 0.08	0.008
Lycopene (μg/ml)	0.23 ± 0.22	0.14 ± 0.07	NS
Vitamin E (μg/ml)	95.3±15	20.7±1.5	0.002

Results are presented as mean ± SEM. *P* value was calculated by the *t*-test.
NS = not significant, ND = not determined, a.u. = arbitrary units

Table 2. Correlations between BMI, plasma carotenoids and markers of inflammation and oxidation

	CRP (mg/L)	IL-6 (pg/ml)	TNF-α (pg/ml)	Dienes (a.u.)
BMI (kg/m2)	*r* = 0.78 *P* < 0.001	*r* = 0.73 *P* = 0.003	*r* = 0.1 *P* = 0.75	*r* = 0.64 *P* = 0.014
Carotenoids (µg/ml)	*r* = -0.37 *P* = 0.19	*r* = -0.62 *P* = 0.02	*r* = -0.32 *P* = 0.26	*r* = -0.63 *P* = 0.02
IL-6 (pg/ml)	*r* = 0.68 *P* = 0.007	– –	*r* = 0.61 *P* = 0.02	*r* = 0.69 *P* = 0.007

Results of obese (after placebo) and lean controls (n=16) by Pearson's correlations.
a.u. = arbitrary units

Table 3. Effect of lyc-o-mato supplementaion on plasma carotenoids and vitamin E in eight obese patients

	Mean difference*	SEM	*P*
Carotenoids (ug/ml)	1.25	0.23	< 0.0001
Lycopene (ug/ml)	0.92	0.21	0.001
Vitamin E (µg/ml)	-35.9	18.1	0.141

*Values after lycopene supplementation compared with values after placebo.
A significant increase ($P < 0.05$) in lycopene and total carotenoids was also shown when values after lycopene supplementation were compared with values at the beginning of study. No significant difference was shown between values after placebo and at the beginning of study.
P was calculated by post-hoc Tukey tests.

Table 4. Effect of lycop-o-mato vs. placebo treatments on markers of inflammation and oxidation products

	At start	After placebo	After lycopene
CRP (mg/L)	6.5 ± 2.4	5.5 ± 2.1	5.6 ± 2.3
IL-6 (pg/ml)	3.6 ± 0.4	3.5 ± 0.6	4.7 ± 1.3
TNFα (pg/ml)	1.4 ± 0.2	1.4 ± 0.2	1.5 ± 0.2
Dienes (a.u.)	181 ± 18	261 ± 29	248 ± 34

Results are mean ± SEM (n=8)
a.u. = arbitrary units

were significant correlations between IL-6 and other markers of inflammation and oxidation (CRP, TNFα and conjugated dienes) [Table 2].

Both total carotenoids and plasma lycopene significantly increased after 4 weeks of Lyc-o-mato supplementation [Table 3]. Concomitantly, vitamin E levels declined but did not reach significance. There were no significant effects of either placebo or lyc-o-mato on plasma glucose, triglycerides, low density and high density-cholesterol (data not shown). Markers of inflammation (CRP, IL-6 and TNFα) and oxidation products (conjugated dienes) did not change throughout the study or upon taking placebo and lyc-o-mato [Table 4]. New correlations appeared only after treatment with lyc-o-mato: plasma lycopene correlated positively with total carotenoids ($r = 0.54$, $P = 0.044$). CRP levels correlated inversely with plasma catorenoids ($r = -0.74$, $P = 0.03$). White blood cells correlated inversely with plasma lycopene ($r = -0.91$, $P = 0.002$) and conjugated dienes correlated inversely with HDL ($r = -0.74$, $P = 0.03$).

DISCUSSION

Our study confirms higher levels of inflammatory markers and oxidative products in obese patients as compared to lean subjects: CRP and IL-6, but not TNFα, were significantly higher among the obese. Previous studies presented correlations between BMI and all three markers of chronic inflammation (CRP, IL-6, TNFα) [11]. Plasma-conjugated dienes also correlated significantly with BMI [12], being nearly eight times higher among the obese in our study.

Similar to other reports, total plasma carotenoids were significantly lower [13], which may result from a low intake, redistribution to adipose tissue and increased consumption, or the combination of these mechanisms in obesity. Plasma carotenoids inversely correlated with IL-6 and conjugated dienes, though not with CRP. An inverse correlation between plasma carotenoids and both IL-6 and CRP has been shown in previous studies [14,15], supporting our hypothesis that carotenoids may interfere with plasma CRP. The inverse correlation between plasma carotenoids and conjugated dienes was also found in previous studies [16]. Four weeks consumption of lyc-o-mato, 30 mg daily, significantly elevated plasma total carotenoids and lycopene among our obese patients. Markers of chronic inflammation and oxidation failed to decrease upon such treatment. Carotenoids have been inversely related to inflammatory markers in epidemiological studies. Thus, we expected CRP to decrease after 4 weeks of treatment. Lower CRP levels were found among healthy men who consumed eight daily servings of fruits and vegetables for 4 weeks compared to two daily servings [17]. Also, diets high or low in carotenoids resulted in a rapid response of plasma carotenoids, but the effect on immune function was delayed, suggesting a slow intracellular carotenoid accumulation [18]. In a recent 2 week study of tomato juice there was a reduction of CRP but not of TNFα and IL-6. An antioxidative effect was present only when vitamin C was added to the tomato juice. Noteworthy was the normal weight of the participants (BMI 21.5 kg/m2, n=24) [19]. Thus, a direct effect of carotenoids on plasma cytokines CRP, IL-6 or TNFα was not reported previously. Some studies demonstrated a significant reduction in plasma-conjugated dienes or their products (malondialdehydes) after 3 weeks supplementation of lycopene or β-carotene [20,21]. Fat tissue redistribution may delay accumulation of carotenoids in intracellular anti-inflammatory target sites. Thus, 4 weeks of supplementation may have failed to modify markers of chronic inflammation due to the short treatment period.

The EURAMIC study, a multicenter case control study, found plasma lycopene levels (and not β-carotene) to be pro-

HDL = high density lipoprotein

tective against acute myocardial infarction, independent of other cardiovascular risk factors [22]. Plasma carotenoids were also inversely correlated with carotoid IMT (intima-media thickness), used to measure atherosclerotic progression [23].

Vitamin E, which was higher among our obese patients, decreased upon lyc-o-mato treatment (though non-significantly). In a cross-sectional study of middle-aged subjects β-carotene was inversely associated with measures of obesity while vitamin E was positively associated with central adiposity [24]. Similar to our observations, a significant decrease in plasma vitamin E levels was found in healthy subjects after supplementation with 8 mg lycopene for 3 weeks. Vitamin E was kept stable in blood lymphocytes. This regime improved protection of DNA from oxidative damage [21]. The decrease in vitamin E in our study may have counterbalanced the beneficial effects on oxidative and pro-inflammatory markers that we expected. Although dietary intake was not recorded, a stable caloric intake and activity were reflected by stable weight measurements throughout the study.

It is well known that many obese populations find it difficult to adhere to lifestyle changes. Our small study of short duration was not able to demonstrate the anti-inflammatory desired effect. Still, carotenoids and other antioxidant-rich foods prevent some of the pro-atherogenic processes that accompany obesity. In support, a recent study investigating the 15 year relationship between carotenoids and markers of inflammation and oxidative stress in a young American population showed a beneficial effect of high plasma carotenoids concerning these markers, including CRP and white blood cells [25]. Further long-term interventional studies are required to determine the protection of different carotenoids against enhanced inflammation and oxidation mediating cardiovascular risk in obesity.

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