Appendix C: Comments provided by LycoRed on Guttenplan *et al.* Study (2001) and Zhao *et al* study (1998)

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המחלקה לביוכימיה קלינית
הפקולטה למדעי הבריאות

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Dr. Dov Hartal
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Dear Dr. Hartal
In response to your request, we have compared the results of the Guttenplan et.al. paper with recent studies that researched the effect of consumption of tomato lycopene on mutagenesis and DNA damage.

Summary of Guttenplan et al. results

The effects of lycopene, fed as a lycopene-rich tomato oleoresin (LTO) was studied at two doses, on in vivo mutagenesis in prostate, colon, and lungs of lacZ mice. Both short-term (2 months) benzo[a]pyrene (BaP)- induced and long-term (9 months) spontaneous mutagenesis were studied (1). The results:
1- Non-significant inhibition of spontaneous mutagenesis in prostate and colon was observed at the higher LTO dose.
2- BaP-induced mutagenesis was slightly inhibited by LTO in prostate. However, enhancement of BaP-induced-mutagenesis was observed in colon and lung.

Points of discussion

1- Is BP a human carcinogen?
BP is clearly a mouse carcinogen which tends to produce tumors at the site of contact (e.g. colon, as in the Guttenplan paper) (2). The relevance of the Guttenplan et al (1) results to human is not clear since it is stated in a IARC monograph (3) that the human data are inadequate to determine carcinogenicity of BP to man. IARC, The International Agency for Research on Cancer is part of the World Health Organization.

2- The method to estimate frequency of mutation (related to DNA damage)
To study mutation accumulation in the DNA of somatic cells and tissues during aging in vivo, a transgenic mouse model has been constructed (4). The same method is effective also for detecting mutation caused by a carcinogen such as BaP. The model harbors plasmid vectors, containing the lacZ reporter gene (a gene present only in bacteria), integrated at various chromosomal locations. Procedures have been worked out to efficiently recovery the plasmids into E. coli host cells, where the frequency of mutation is determined. A positive selection system, permitting only E. coli cells with a lacZ mutated plasmid to grow, allows for the determination of mutation frequencies as the ratio of mutant colonies versus the total number of plasmid copies recovered. Some studies assessed the inhibitory activities of chemopreventive agents such as 1,2-dithiole-3-thione, conjugated linoleic acids, tea, curcumin, chlorophyllin-chitosan, and sulindac in the lacZ model (5).

3- Lycopene preparation.
There is no information about other components of the LTO, in addition to the carotenoids. There is also no information on the toxicity of the preparation.

Recent studies which report either protection or enhancement of DNA damage by tomato or lycopene preparation.

Studies which reported enhancement of DNA damage.

1- Animal study. Guttenplan paper (1).

2- Animal study. The potential beneficial or adverse affect of prolonged dietary administration of moderate to high doses (1-100 mg/kg diet) of the antioxidants, lycopene, quercetin and resveratrol or a mixture of lycopene and quercetin was investigated in male F344 rats. In this model all antioxidants and the antioxidant combination significantly increased the level of lymphocytic DNA damage (6).

Studies which did not find an effect on DNA damage.

1- Bacteria. The effects of beta-carotene, canthaxanthin, and extracts of tomato paste (containing lycopene) and orange juice (containing cryptoxanthin) on aflatoxin B1 (AFB1)-induced mutagenesis in the bacteria S. typhimurium were investigated (7).
Each experimental carotenoid, except lycopene, inhibited AFB1-induced mutagenesis. It is not clear whether the bioavailability of all tested preparations was the same.

2- Human and In vitro study. DNA damage (frequency of single-strand breaks-SSB, comet assay) was analyzed in human peripheral blood lymphocytes from healthy male volunteers supplemented with lutein, beta-carotene or lycopene (natural isolate capsules, 15 mg/d, 4 weeks) (8). Lycopene and lutein had no effect on DNA damage in control lymphocytes or following oxidative challenge. However, increased plasma beta-carotene was associated with more DNA damage in control cells. In an in vitro system the higher concentration of lycopene or lutein increased DNA damage in control cells whereas the lower carotenoid concentrations were protective under oxidative challenge.

3- Animal study. The in vivo effects of carotenoids on AFB1-induced liver DNA damage were evaluated using DNA single-strand breaks (9). In contrast to lycopene or to an excess of vitamin A, both of which had no effect, beta-carotene, and other tested carotenoids, were very efficient in reducing AFB1-induced DNA single-strand breaks.

Studies which reported inhibition of DNA damage.

1- Human study. The effect of carotenoid-rich foods on DNA damage (comet assay) was studied in healthy male subjects (10). The supplementation of the diet with tomato, carrot or spinach products resulted in a significant decrease in endogenous levels of strand breaks in lymphocyte DNA.

2- Human study. Lymphocyte lycopene concentration and DNA protection from oxidative damage is increased in women after a short period of tomato consumption (11).

3- In vitro. Effects of the dietary antioxidants ascorbate, alpha-tocopherol, (-)-epigallocatechin gallate (EGCG) and lycopene on spontaneous mutagenesis were studied in a special cell model which exhibit elevated levels of spontaneous mutagenesis (12). The strongest antimutagenic compound was lycopene.

4- Human study. Spinach and tomato consumption increases lymphocyte DNA resistance to oxidative stress (13).

5- Human study. Antioxidant levels in the blood of healthy males in Bratislava was found to vary according to season (14). DNA damage, as analyzed in lymphocytes using the comet assay, was lower in summer than in winter. This was correlated with lycopene blood levels which peaked in the summer.

6- Animal study. Inhibitory effects of lutein and lycopene on hepatic DNA strand breakage in a rat model of liver carcinogenesis (15). Hepatic DNA strand breakage evaluated by the comet assay was lower in carotenoid-treated animals when compared with the control group

7- Animal study. The effect of lycopene supplementation on lymphocytes and liver DNA damage (comet assay) was studied in rats (16). Compared with control, the damage of DNA in the lycopene group was reduced significantly.

8- Human study. The effect of tomato products consumption on lymphocytes DNA damage (comet assay) was studied in healthy female subjects (17). There was an improved protection from DNA oxidative damage.

9- Human study. Intervention with tomato oleoresin extract resulted in significant increases in total plasma lycopene and resulted in decreased levels of DNA damage (18).

10- Human study. The effect of lycopene supplementation on DNA damage was studied in men with prostate cancer (19). Leukocyte and tissue 8-OH-deoxyguanosine DNA content was measured to estimate DNA damage. Leukocyte and prostate tissues from tomato sauce-supplemented patients had lower 8OHdG compared with the control group.

11- Human study. The effects of consumption of tomato sauce-based pasta dishes on lycopene uptake, and oxidative DNA damage, in patients already diagnosed with prostate cancer was analyzed (20). Compared with preintervention levels, leukocyte oxidative DNA damage was statistically significantly reduced after the intervention.

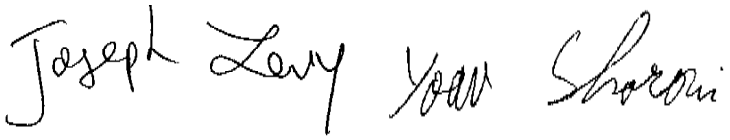
12- Human study. The capacity of lymphocytes isolated from volunteers supplemented with beta-carotene, lutein or lycopene to recover from DNA damage induced in vitro by treatment with H_2O_2 was analyzed (21). In those individuals who showed increases in lycopene concentrations, the recovery was significantly faster.

13- In vitro study. The effect of lycopene on the formation of 8-oxo-dGuanosine (a marker for DNA damage by oxidation) in monkey cells exposed to ferric nitrilotriacetate was analysed (22). Lycopene supplementation decreased by 77% the 8-oxodGuo levels in Fe-NTA/ascorbate-treated cells.

14- Animal study. The effect of lycopene pretreatment on oxidative damage to DNA, in liver of animals subjected to intraperitoneal ferric nitrilotriacetate administration was studied (23). Five days of lycopene pretreatment almost completely prevented liver oxidative DNA damage.

15- Human study. The study correlate the levels of 8-OHdG, a marker of oxidative damage to DNA, in peripheral lymphocytes of Alzheimer disease patients and the plasma levels of various antioxidant including lycopene (24). Lymphocyte DNA 8-OHdG content was significantly higher and plasma levels of antioxidants (with the exception of lutein) were significantly lower in patients with Alzheimer disease compared with controls.

In summary, the results of Guttenplan et al and Breinholt et al should be considered in the context of 20 studies, known to us, on the effect of various lycopene (or tomato) preparations on DNA damage. These two papers which presented some negative effects of lycopene were done with animals. It is important that the Guttenplan's results were obtained where mutations resulted from a strong challenge with a carcinogen. In the spontaneous setup which is closer to real life, lycopene was protective. In three other papers there was no effect of lycopene. In the majority (15 papers) a protective effect of lycopene on DNA stability was reported. The majority of these studies were performed in humans.



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Additional Comments on the Gluttenplan paper

Further investigations have shown that the test substance used in the study reported by Gluttenplan et al (2001) is very dissimilar to the lycopene oleoresin from tomatoes provided by Lyc-O-Mato®.

Reference is made in the Gluttenplan paper to the composition of the test material being the same as that used by Zhao et al (1998). In fact two authors are common to both papers.

Zhao (1998) gives more detail of the composition. The material used was provided by Cognis USA under the trade name 'Betatene' which is a carotenoid concentrate suspended in a medium chain triglyceride which is an added carrier and not a natural tomato component In comparison Lyc-O-Mato is a 100% natural tomato extract and the phytonutrients are suspended in the tomato oil.

The carotenoid composition for Betatene is given as:

	% Total Carotenoids
Lycopene	66
ß-carotene	22
Phytofluene	6
Phytoene	5
Zeta-carotene	0.7
2; 6-cyclolycopene 1, 5 diol	0.7

From this information it appears that the beta carotene / lycopene ratio (1 : 3) is very considerably higher by a factor of 10 than that found in tomatoes and in Lyc-O-Mato® (0.1 : 3). The 1 : 3 ratio is unlikely to be obtained only from a tomato source and it is likely that the Betatene is a mixture of carotenoids and not the lycopene-rich tomato oleoresin referred to in the Guttenplan and Zhao papers.

The high proportion of beta carotene in the test material (when compared with tomato oleoresin) could have a confounding effect and the pro-oxidant effects cannot necessarily be attributed solely to lycopene, as beta carotene is known to have a greater pro-oxidant effect when compared to lycopene and tends to be better absorbed than lycopene. According to the Zhao paper the level of beta carotene in the liver was only 10% of the lycopene level but retinol formed from the beta carotene was 10 times higher than lycopene.

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