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Promotion of the Elimination of Lactic Acid in the Blood after Exercise by Ingestion of Citric Acid or Lemon Juice

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1. Introduction

Citric acid is the source of sourness in lemons and accounts for 6% of lemon juice ¹⁾. In addition, citric acid is a main intermediary metabolite in energy metabolism in the body, and researchers have been interested in the possible effects of administration of citric acid on metabolism. It has been reported that exercise increases the activity of enzymes synthesizing citric acid in muscle, resulting in an increase in citric acid concentration ²⁾. This fact suggests the possibility that administration of citric acid before, during or after exercise may affect energy metabolism in relation to exercise.

Previous reports have shown that administration of citric acid to human subjects before short-term (several minutes) exercise results in improved performance through prevention of a decrease in blood pH during exercise and other effects ³⁻⁵⁾. In addition, it has been reported that concomitant administration of citric acid and glucose to rats after depletion of glycogen in the liver and muscle through endurance exercise promotes a quicker recovery of glycogen in both types of tissues than the administration of glucose alone ⁶⁾. However, there has been no report on the possible effects of citric acid on human metabolism when ingested after endurance exercise. Therefore, we examined the possible effects of citric acid and lemon juice on human blood components after exercise ⁷⁾.

2. Experiments examining possible effects of citric acid or lemon juice administered to human subjects ⁷⁾

Six healthy male adults with experience in athletics (between 25 and 30 years of age) served as experimental subjects. Each subject participated in all of the following three experiments: after taking exercise on the day of experiment, ingesting (1) a test solution containing citric acid and glucose, (2) a test solution containing lemon juice and glucose, or (3) a test solution containing glucose (control). At 9:00 a.m. on the day of the experiment, the subjects were given some bananas (equivalent to 200 kcal [230 g] per 65 kg b.w.) in order to prevent a low blood sugar concentration during exercise. One hour later (at 10:00 a.m.) the subjects exercised using ergometers. The exercise strength load and time was 50% of the maximum strength load for 15 min. followed by 70% of the maximum strength for 45 min. Immediately after the exercise, the subjects ingested 500 ml of either (1) the test solution containing citric acid (0.4 g/kg b.w.) and glucose (1.5 g/kg b.w.), (2) the test solution containing lemon juice (equivalent to 0.4 g/kg b.w. as citric acid) and glucose (1.5 g/kg b.w.), or (3) the test solution containing glucose and containing the same calories as the other two test solutions (containing the same amount of glucose as the other two test solutions and adjusted for the calories of citric acid using fructose ⁶⁾). The subjects were allowed a 60-minute rest after the exercise.

3. Results and discussion

Lactic acid is a compound synthesized by glycolysis in muscle and is known to be associated with muscle fatigue. Fig. 1 shows the change in lactic acid levels in the blood before and after the exercise and during the one-hour rest in the present study. In all groups of subjects who ingested one of the test solutions, the lactic acid level in the blood at rest was approximately 1 mM, which increased to 4.0

mM or higher after 1 hour exercise. Although blood lactic acid levels decreased gradually during the rest period in all groups, the levels decreased faster in the groups which ingested the test solutions containing “citric acid and glucose” or “lemon juice and glucose” than in the group which ingested the control test solution containing glucose; at the end of the 60-min. rest, the levels for the lemon juice group and citric acid group were significantly lower than the level for the control group. On the other hand, the serum citric acid levels at the end of the 60-min. rest were significantly increased in the citric acid group (50.5 µg/ml) and the lemon juice group (46 µg/ml) compared to that in the control group (22.3 µg/ml). These results suggest that the elimination of lactic acid in the blood is promoted by ingestion of citric acid or lemon juice after exercise.

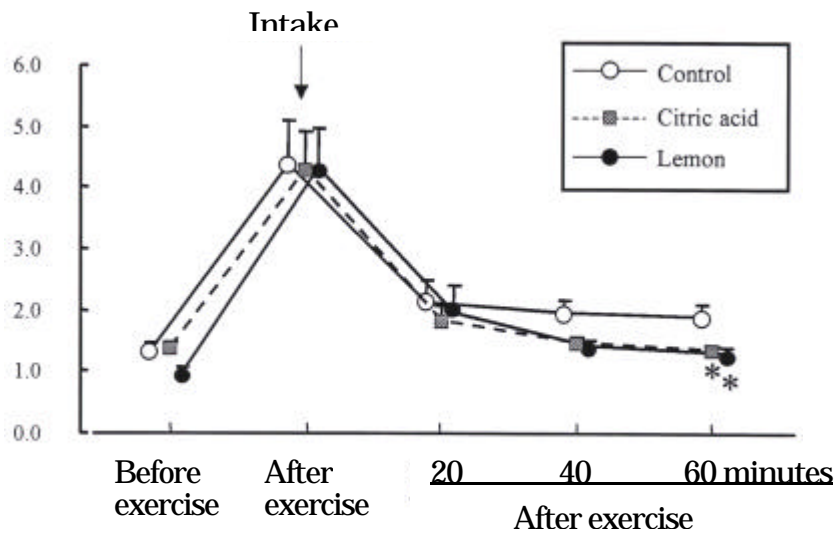


Fig.1: Effect of Citric acid and Lemon juice on Blood Lactic acid concentration before /after exercise. * $p < 0.05$

Researchers have long been interested in the possible effects of citric acid on muscle fatigue, but a possible effect of citric acid on lactic acid in the blood has not been examined. The promotion of the elimination of lactic acid in the blood by the ingestion of citric acid found in the present study may be due to inhibition of the release of lactic acid from muscle, promotion of the disposal of lactic acid in the liver, or both. The effects of citric acid in the body may be speculated as follows:

Citric acid increases the activity of acetyl-CoA carboxylase, an enzyme transforming acetyl-CoA into malonyl-CoA⁸⁾, which suggests the possibility that the administration of citric acid may promote the synthesis of malonyl-CoA (Fig. 2). Malonyl-CoA inhibits the uptake of fatty acids into mitochondria because malonyl-CoA strongly inhibits carnitine-palmitoyltransferase I in mitochondria⁸⁾.

On the other hand, pyruvate dehydrogenase complex (PDC) in mitochondria is known to be inactivated under conditions in which the fatty acid oxidation is promoted and activated when the oxidation is inhibited⁹⁾. Therefore, it is expected that when synthesis of malonyl-CoA is promoted, the activity of PDC will increase, resulting in that oxidation of pyruvic acid and lactic acid is promoted. In addition, citric acid is an inhibitor of phosphofructokinase, a regulating enzyme in the glycolytic pathway (Fig. 2), and therefore inhibits the breakdown of glucose. These two effects of citric acid may be responsible for the promotion of the breakdown of lactic acid after administration of citric acid. However, more research is needed to verify this hypothesis.

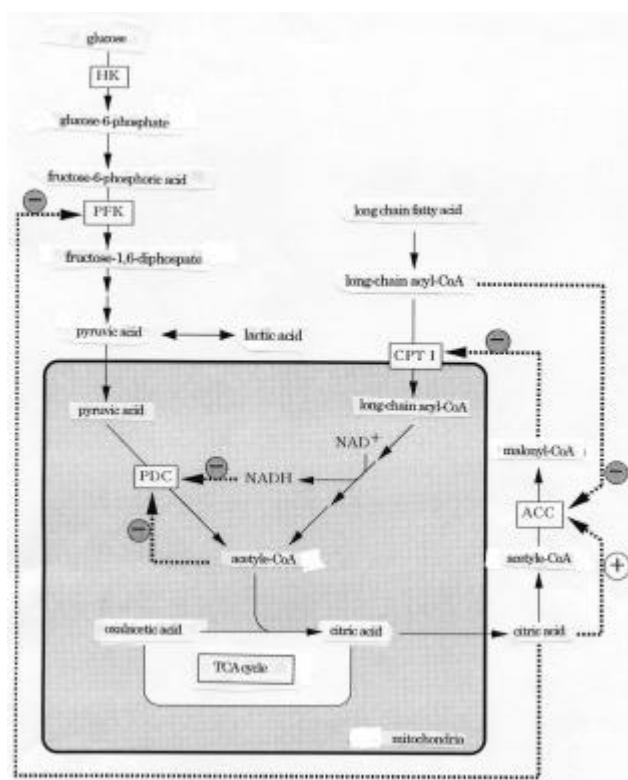


Fig.2 Effect of Citric acid for oxidation of Glucose and Fatty acid

HK: , PFK: phosphofructokinase, PDC: pyruvate dehydrogenase complex, CPT?: carnitine-palmitoyltransferase ?, ACC: acetyl-CoA carboxylase

⊖ : inhibition, ⊕ : promotion

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Citric Acid and Improvement in Blood Fluidity

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1. Introduction

The underlying condition of thrombotic disease is lack of elasticity in blood vessels due to arteriosclerosis, which, together with increased platelet coagulation, decreased red blood cell deformability, increased adhesiveness of white blood cells and other factors, is considered to result in decreased blood fluidity. It is widely known that eicosapentaenoic acid (EPA) found in such fish as sardines is effective in improving blood fluidity. On the other hand, we have already reported that blood fluidity is also improved by such vegetables and vegetable products as onion, sweet peppers and cacao mass. Here we present a study on the effects of lemon juice on blood fluidity.

2. Methods

2.1 Oral dose experiment (in vivo)

• Subjects and materials

The subjects were 13 healthy male and female adults aged between 30 and 43 who gave informed consent to this study.

A twofold dilution of 30 mL of Pokka 100 Lemon (100% lemon juice; Pokka Corporation) was used.

• Measurement of blood fluidity (whole blood passage time)

Before ingesting the test solution, blood was collected from the subjects' brachial vein with heparin (blood:heparin=9.5 ml:0.5 ml). Blood was collected again one and three hours after the ingestion of the test solution.

Using blood filter tips (Bloody 6-7) of 7 μm in width, 30 μm in length and 4.5 μm in depth arranged in a line of 8736 tips, the whole fresh blood obtained was flowed in a cell microreology measurement instrument (MC-FAN; Hitachi Haramachi Electronics) with the difference of 20 cm in water column and measured the passage time of 100 μl of blood. Before measuring the passage time of the whole blood, that of 100 μl of saline was measured and whole blood passage time was obtained by adjusting the measurements to the measured passage time for saline in 12 seconds using the following formula:

(blood passage time) \times 12 sec./ (saline passage time)

• Measurement of the effect on the inhibition of platelet coagulation

A Hematracer (Nikko Bioscience) was used for the measurement of platelet coagulation. The whole blood collected before the ingestion of the test solution was centrifuged at 1,000 rpm for 10 min and the platelet suspension (platelet rich plasma, PRP) obtained as the supernatant was placed in a cuvette and this was regarded as 0% in transmittance. The lower layer was further centrifuged at 3,000 rpm for 15 min at room temperature, and the platelet poor plasma (PPP) obtained as the supernatant was used as the standard value for 100% transmittance. For measurement, as an inducer of platelet coagulation 20 μl of collagen at various concentrations were added to 200 μl of PRP while being stirred at 37 $^{\circ}\text{C}$. The collagen concentrations of the samples were determined by examining collagen concentrations high enough to induce platelet coagulation in 80% to 100% of platelets.

Similarly, PRP and PPP were prepared from the whole blood collected one and three hours after the ingestion of the test solution, followed by the addition of 20 µl of collagen at the same concentrations as those examined for the samples collected before the ingestion of the test solution, and the inhibitory rates were obtained from the platelet coagulation curves for the samples before and after the ingestion of the test solution using the following formula:

$$\text{Inhibitory Rate} = (\text{Ac}-\text{As}) / \text{Ac} \times 100$$

(Ac= coagulation rate before the ingestion of the test solution; As= coagulation rate after the ingestion of the test solution)

2.2 In vitro test

• Test solutions

In addition to the aforementioned lemon juice, citric acid, vitamin C and eriocitrin, which are physiologically active components of lemon juice, were added to the samples at the ratio of the concentrations corresponding to that found in lemon juice (the ratio of the concentrations found in 10,000 folds dilution of lemon juice) and examined. The final concentrations of these components in the blood samples were: citric acid, 6 ppm; vitamin C, 0.04 ppm; and eriocitrin, 0.02 ppm.

• Measurement of blood fluidity (in a threefold blood dilution)

Blood was collected from the subjects' brachial vein in the same manner as the oral dose experiment. A threefold dilution of the blood was prepared using the PPP of respective subjects. 10 µl of the test solutions at adjusted concentrations were added to 1 ml of the threefold blood dilution.

• Measurement of the inhibition of platelet coagulation

In the same manner as the oral dose experiment, PRP and PPP were prepared from the whole blood obtained from the subjects and the collagen concentrations of the samples were determined using the PRP samples.

For measurement, each test solution was added to PRP stirred at 37 °C, followed by the addition of 20 µl of collagen as an inducer of platelet coagulation, and the inhibitory effect of the test solution on platelet coagulation was determined by examining the resulting platelet coagulation.

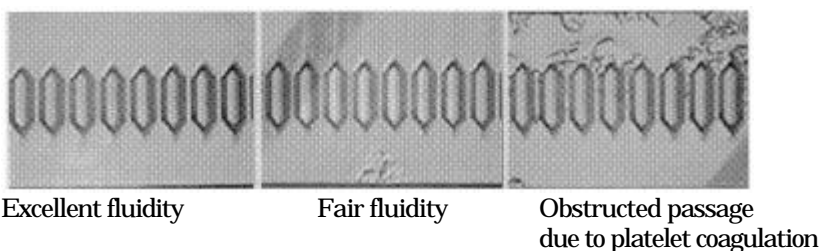


Fig.1 Conditions of blood fluidity Excellent fluidity

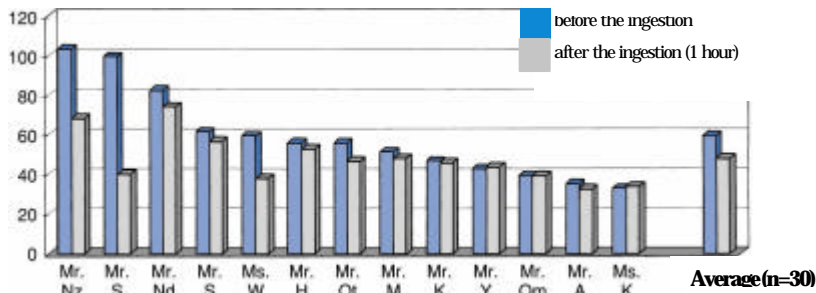


Fig.2 Improvement in blood fluidity by the ingestion of 30 ml of lemon juice (in twofold dilution) (in vitro)

3. Results

The whole blood passage time varied among the thirteen subjects and ranged between 33.3 sec/100 μ l and 100.0 sec/100 μ l. Those who showed passage obstruction due to clotting caused by platelet coagulation showed improvement one hour after the ingestion of the test solution. In 11 out of 13 subjects the blood passage time decreased, showing improvement in blood fluidity with an average improvement rate of 13.9% (Fig. 2). As a whole, in the oral dose study the subjects with longer blood passage time showed more improvement in blood fluidity. One subject showed an improvement rate as high as 59.7%. As for platelet coagulation, the ingestion of the test solution resulted in an average improvement rate of 45.2%.

In order to determine which component of lemon juice is effective in improving blood fluidity, citric acid, vitamin C and eriocitrin were added to the blood samples and the blood passage time was measured. The results show improvement for all components (Fig. 3), but citric acid was the strongest in activity, followed by eriocitrin and vitamin C (Fig. 4). Citric acid is the main component causing the sourness of lemons, which contain citric acid at one of the highest levels found in foods. Eriocitrin and vitamin C showed high inhibitory rates as well.

As part of the mechanisms of platelet coagulation, it is known that prostaglandin I₂ and thromboxan A₂ produced through the EPA cascade show inhibitory effects on platelet coagulation. On the other hand, in the metabolic process of arachidonic acid, strong inducers of platelet coagulation such as prostaglandin I₂ (PGI₂) or thromboxan A₂ (TXA₂) are produced by cyclooxygenase. The results of the present study suggest that lemon juice, specifically citric acid, inhibits the activity of cyclooxygenase in the arachidonic acid cascade and decreases the production of PGI₂ and TXA₂, resulting in the inhibition of platelet coagulation and improvement in blood fluidity.

*The present study was a collaborative study with Pokka Corporation.

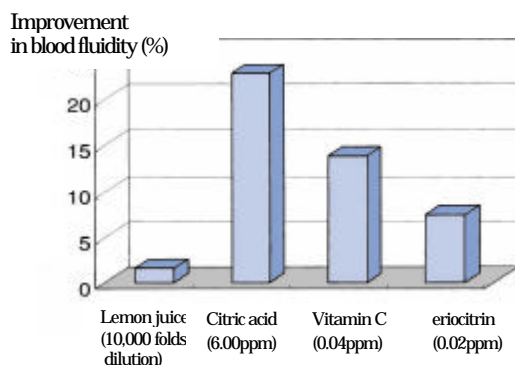


Fig.3 Improvement in blood fluidity by lemon juice and its components (in vitro)

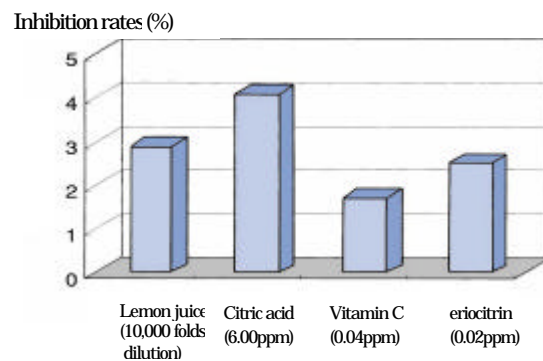


Fig.4 Platelet coagulation inhibition rates

Impact of Folic acid Fortification of the US Food Supply On the Occurrence of Neural Tube Defects

(Honein et al, JAMA 2001; 285: 2981-2986)

Daily consumption of 400 µg of folic acid before conception and during early pregnancy dramatically reduces the occurrence of neural tube defects (NTDs). Before food fortification, however, only an estimated 29% of US reproductive-aged women were taking a supplement containing 400 µg of folic acid daily. The US Food and Drug Administration authorized addition of folic acid to enriched grain products in March 1996, with compliance mandatory by January 1998.

Objective: To evaluate the impact of food fortification with folic acid on NTD birth prevalence.

Period and Subjects: National study of birth certificate data for live birth to women in 45 US states and Washington, DC, between January 1990 and December 1999.

Results: The birth prevalence of NTDs reported on birth certificates decreased from 37.8 per 100,000 live birth before fortification to 30.5 per 100,000 live birth conceived after mandatory folic acid fortification, representing a 19% decline.

During same period, NTD birth prevalence declined from 53.4 per 100,000 to 46.5 per 100,000 for women who received only third-trimester or no prenatal care. Third-trimester terminations are rare, even with a prenatal diagnosis of an NTD. Therefore, affected pregnancies without obstetric oversight in the first 2 trimesters are unlikely to be terminated.

Table: Effect estimates for the observed decline in NTDs following US folic acid fortification of the grain supply

	Total NTD PR (95% CI)	Spina Bifida PR (95% CI)	Anencephaly PR (95% CI)
All live birth			
After fortification ^{*1}	0.81 (0.75-0.87)	0.77 (0.70-0.84)	0.89 (0.78-1.01)
Before fortification ^{*2}	1.00	1.00	1.00
Third-trimester			
After fortification ^{*1}	0.87 (0.64-1.18)	0.71 (0.47-1.07)	1.14 (0.71-1.83)
Before fortification ^{*2}	1.00	1.00	1.00

*1: Oct. 1998 – Dec. 1999 *2: Oct. 1995 – Dec. 1996

PR: Prevalence ratio, CI: confidence interval

A new recommended dietary allowance of vitamin C for healthy young women

(Mark Levine et al, PNAS Vol.98 No.17, 9842-9846)

The recently released Recommended Dietary Allowance of vitamin C for women, 75mg daily, was based on data for men. We now report results of a depletion-repletion study with healthy young women, using vitamin C doses of 30-2,500 mg daily. The relationship between dose and steady-state plasma concentration was sigmoidal. Only doses above 100 mg were beyond the linear portion of the curve. Plasma and circulating cells saturated at 400 mg daily, with urinary elimination of higher doses. Biomarkers of endogenous oxidant stress, plasma and urine F2-isoprostanes, and urine levels of a major metabolite of F2-isoprostanes were unchanged by vitamin C at all doses, suggesting this vitamin does not alter endogenous lipid peroxidation in healthy young women. By using Food and Nutrition Board guidelines, the data indicate that the Recommended Dietary Allowance for young women should be increased to 90 mg daily.

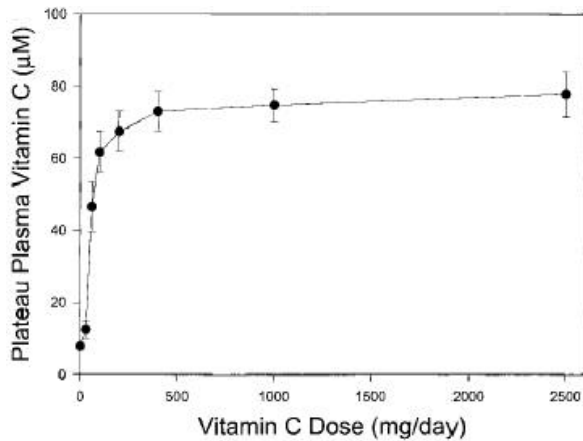


Fig.1 Steady-state vitamin C concentrations in plasma for subjects as a function of all doses

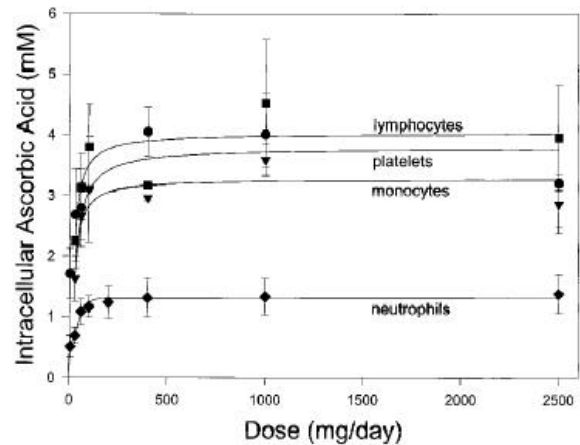


Fig.2 Intracellular vitamin C concentrations in circulating cells as function of dose.

Recycling of the Ascorbate Free Radical by Human Erythrocyte Membranes

(James M et al, Free Radical Biology & Medicine, Vol.31 No.1117-124 2001)

Reduction of the ascorbate free radical (AFR) at the plasma membrane provides an efficient mechanism to preserve the vitamin in a location where it can recycle α -tocopherol and thus prevent lipid peroxidation. Erythrocyte ghost membranes have been shown to oxidize NADH in the presence of the AFR. We report that this activity derives from an AFR reductase because it spares ascorbate from oxidation by ascorbate oxidase, and because ghost membranes decrease steady-state concentrations of the AFR in a protein- and NADH-dependent manner. The AFR reductase has a high apparent affinity for both NADH and the AFR ($< 2 \mu\text{M}$). When measured in open ghosts, the reductase is comprised of an inner membrane activity (both substrate sites on the cytosolic membrane face) and a trans-membrane activity that mediates extracellular AFR reduction using intracellular NADH. However, the trans-membrane activity constitutes only about 12% of the total measured in ghosts. Ghost AFR reductase activity can also be differentiated from NADH-dependent ferricyanide reductase(s) by its sensitivity to the detergent Triton X-100 and insensitivity to enzymatic digestion with cathepsin D. This NADH-dependent AFR reductase could serve to recycle ascorbic acid at a crucial site on the inner face of the plasma membrane.

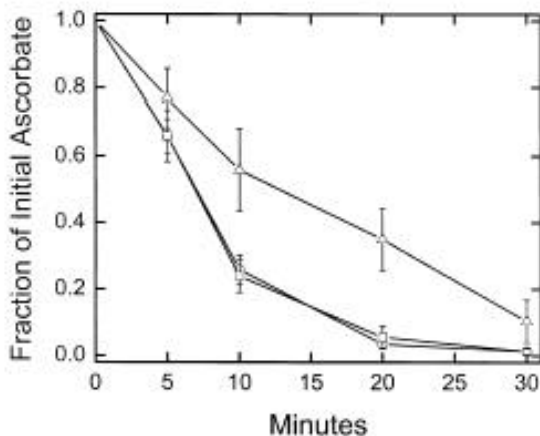


Fig.1 Sparing of ascorbate by ghost membrane in the presence of NADH.

*Ascorbate (50 mM) and ascorbate oxidase (40 mU/ml) were incubated at 37°C in 5P7.4 that contained 0.4 mM NADH (circles), 0.4 ml packed ghosts/ml (1.5 mg/ml) (squares), or NADH plus ghosts (triangles).

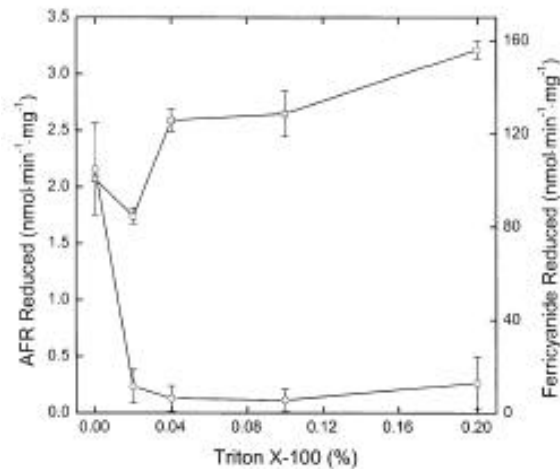


Fig.2 Effects of Triton X-100 on leaky ghost NADH-ferricyanide reductase activities.
*AFR reduced: circles
Ferricyanide reductase: squares

Oxygenated Carotenoid Lutein and Progression of Early Atherosclerosis

(James H. Dwyer et al, Circulation.2001;103: 2922-2927)

Following three studies (Epidemiology, Coculture, Mouse model) presents evidence that high intake of lutein may protect against the development of atherosclerosis.

1. Epidemiological study

Progression of intima-media thickness (IMT) of the common carotid arteries over 18 months was determined ultrasonographically and was related to plasma lutein among a randomly sampled cohort of utility employees age 40 to 60 years (n=480). IMT progression declined with increasing quintile of plasma lutein (P for trend=0.007, age-adjusted; P=0.0007, multivariate). Covariate-adjusted IMT progression (mean \pm SEM) was 0.021 \pm 0.005 mm in the lowest quintile of plasma lutein, whereas progression was blocked in the highest quintile (0.004 \pm 0.005 mm; P=0.01).

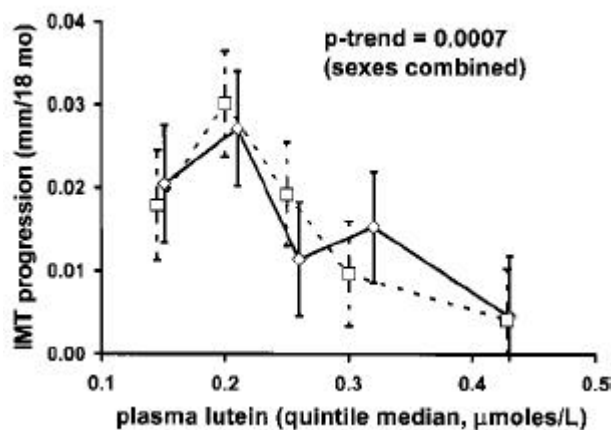


Figure 1. Inverse relation between change in carotid IMT and quintiles of plasma lutein.

? : men (n=248), ◇: women (n=214)

2. Coculture

The impact of lutein on oxidative modification of LDL was evaluated in a coculture model of the artery wall formed from endothelial and smooth muscle cells from human aorats.

The impact of lutein on the monocyte chemoattract property of LDL incubated with the coculture was evaluated in 2 types of chemotaxis assays.

A. The coculture was incubated with different concentrations of lutein overnight. Cells were then washed and coincubated with constant concentrations of LDL for 8 hours.

B. LDL was first incubated with different concentrations of lutein for 4 hours and then added to the coculture for 8 hours.

Results: A dose-dependent reduction in chemotaxis for monocytes is apparent for increasing concentrations of lutein in each of the experiments. A dramatic inhibitory effect of lutein on chemotaxis occurs with pretreatment of the coculture cells. Note that lutein at 100 nmol/L inhibits monocyte migration at a level similar to that observed for human HDL.

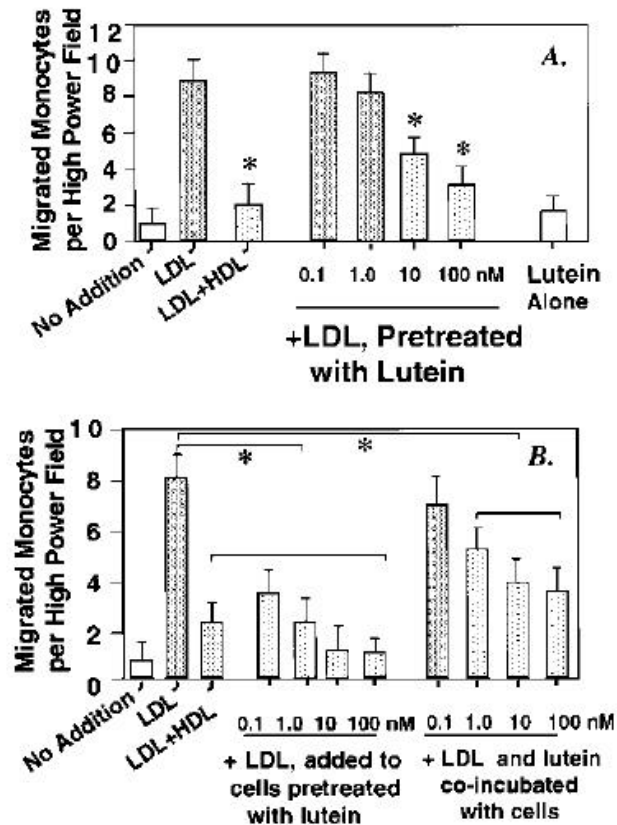


Figure 2. Effect of pretreatment with lutein on LDL oxidation and resulting monocyte chemotactic activity. *p<0.05

3. Mouse model

In the mouse models, using apoE-null mice and LDL receptor-null mice, supplementation with lutein very markedly reduced the atherosclerotic lesions.

<Apo-E-null Mouse>

Control (chow diet): n=7

Chow diet + Lutein (0.2%): n=9

Atherosclerotic lesion formation of aortic arch was assessed after 8 weeks: Lesion size was reduced by 44% in the lutein condition ($5.5 \pm 1.5 \times 10^6 \mu\text{m}^2$) relative to control ($9.9 \pm 1.1 \times 10^6 \mu\text{m}^2$).

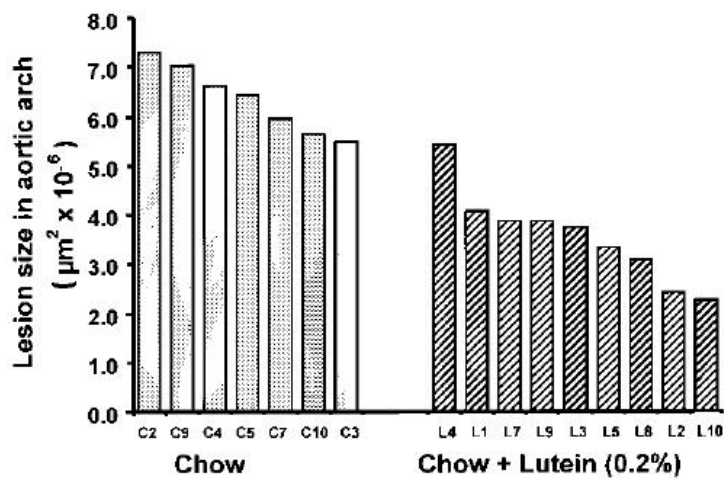


Figure 3. Aortic lesion size in ascending segment of aortic arch among apoE-null mice.

<LDL Receptor-Null Mouse>

Procedures for the LDL receptor-null mouse experiments were identical to those for the apoE-null mouse, except for the diet. (Western diet and Western + Lutein 0.2%)

Lesion size was reduced by 43% in the lutein supplemented condition ($22 \pm 10 \times 10^6 \mu\text{m}^2$) relative to controls ($38 \pm 5 \times 10^6 \mu\text{m}^2$).