

Grapefruit juice and serum lipids in healthy adults

Christina Jonsson and Lars Ellegård

Department of Clinical Nutrition, Sahlgrenska Academy at Göteborg University, Göteborg, Sweden

Abstract

Background: Grapefruit juice has been reported to decrease serum cholesterol in patients with hyperlipidaemia. Whether grapefruit juice can influence serum lipids in healthy people had not been investigated.

Objective: To investigate the effect of grapefruit juice on serum lipid levels in healthy adults by comparing grapefruit juice with apple juice in the same individuals.

Design: In a cross-over study, 28 healthy volunteers took 250 ml grapefruit juice or apple juice daily for 3 weeks followed by a 3 week washout, and then the juices were swapped for the second intervention period. Serum samples for total, low-density lipoprotein (LDL)-, high-density lipoprotein (HDL)-cholesterol and triglycerides were collected before and after each period, and analysed batchwise after the study.

Results: In the grapefruit juice period LDL-cholesterol decreased by 6% ($p=0.04$). Serum lipids were not significantly affected by apple juice. There were no differences in lipid levels between grapefruit juice and apple juice periods. During the whole 9 week study total and LDL-cholesterol decreased by 8% ($p<0.0001$) and 14% ($p<0.0001$), respectively, whereas HDL-cholesterol increased by 6% ($p=0.028$).

Conclusions: Consumption of grapefruit juice decreased LDL-cholesterol by 6%, but with no significant differences compared with the apple juice or washout periods. Thus, the hypocholesterolaemic effect of grapefruit juice cannot be separated from the general time effect encountered in this study.

Keywords: *apple juice; cholesterol; cross-over; grapefruit juice; HDL; LDL; triglycerides*

Received: 4 May 2006; Revised: 10 Jul. 2006; Accepted: 8 Aug. 2006

Introduction

Coronary heart disease (CHD) is the leading cause of death in the Western world. CHD is caused by atherosclerosis, a process characterized by endothelial dysfunction. Risk factors for atherosclerosis include high serum cholesterol and serum triglycerides (1). The target level for serum cholesterol in Sweden is $<5.0 \text{ mmol l}^{-1}$ (www.mpa.se/workshops/reko/030613lipid.shtml). Diet influences both serum cholesterol and serum triglycerides. Dietary fats play a major role in CHD development, mostly by modulating plasma lipoprotein concentrations. In addition, other components, such as meal pattern, total energy intake, dietary fibre, sugars and alcohol, influence the serum lipids. Current Nordic nutrition recommendations promote the consumption of more fruit, vegetables and berries (2). Fruit juices contain micronutrients, antioxidants such as flavonoids and dietary fibre (2). Dietary fibre has been reported to increase faecal sterol excretion and to decrease serum cholesterol levels, with more pro-

nounced effects from soluble dietary fibre (3). Some micronutrients and flavonoids act as antioxidants (2) and grapefruit juice is rich in antioxidants (4), while the antioxidant capacity of apple juice is less pronounced (5). However, neither antioxidants in the diet nor antioxidant supplements such as β -carotene, vitamin E and vitamin C have been conclusively shown to influence serum cholesterol (6, 7). Animal studies on the effects of fibre supplements from apple juice on serum lipids have been disappointing (8), while reports on grapefruit juice have been both positive and negative (9, 10). Apple juice does not influence serum lipids in humans (11). Grapefruit juice has recently been reported to decrease serum cholesterol by 16% and low-density lipoprotein (LDL)-cholesterol by 21% compared with mineral water in patients with hyperlipidaemia (12). Whether grapefruit juice can influence serum lipids in more normolipaeamic subjects is unknown. The aim of this study was to investigate the effect of grapefruit juice on levels of serum lipids in healthy subjects by comparing

grapefruit juice and apple juice in the same individuals.

Methods

Subjects

Thirty subjects (15 men and 15 women) volunteered for the study, which lasted for 9 weeks. Two subjects withdrew (one subject after 3 weeks, owing to changed working hours, and another after 8 weeks, owing to pregnancy). Clinical data on the remaining 28 subjects are shown in Table 1. The subjects were healthy adults, aged >18 years, with no noted hyperlipidaemia. Exclusion criteria were pregnancy and prescription drugs. Diet, dietary supplements, physical activity and serum lipids levels are representative for the Swedish population (13). Informed consent was obtained from each participant.

Study design

The study had a cross-over design with two intervention periods (each of 3 weeks) and a 3 week washout period. Subjects were allocated by number of inclusion to two groups (A and B) of 15 participants each. Group A started with 250 ml grapefruit juice daily for 3 weeks, whereas group B started with the same amount of apple juice. Fasting blood samples for total cholesterol (TC), LDL-cholesterol, high-density lipoprotein (HDL)-cholesterol and triglycerides were collected on four occasions, before and after each period. Body weight was recorded before and after each period. The subjects were advised to keep their diet and activity habits unchanged. If the subjects usually consumed juice, they could continue to do so during both the two intervention periods and washout. The study protocol was approved by the regional

ethics committee in Göteborg on 4 April 2005 (registration no. 548-04).

Diet and activity questionnaire

On the first visit, the subjects completed a self-administered questionnaire on their habitual diet, dietary supplements and physical activity, including meal patterns, fat, milk, fruit and fruit juice, and a food-frequency questionnaire concerning roots, vegetables, fruit, coarse bread, sandwiches, oats, nuts/almonds, salmon/mackerel/Baltic herring, fried food, alcohol, cheese, cream, buns/cookies, snacks, sweets/chocolate/ice-cream, pizza/hamburger, and syrup/soft drinks. The eight frequency categories used were: never, 1, 2 times per month; 1, 2–3 times per week; 1, 2–3, ≥ 4 times per day. During the other study visits, the subjects also reported consumption of juices, any illness, any changes in diet or physical activity, and compliance.

Juices

The grapefruit juice was 100% pure squeezed juice. The apple juice was reconstituted from juice concentrate by the manufacturer. Both juices were commercially available in Sweden. The composition of the juices is shown in Table 2.

Blood sampling and analyses

Blood was drawn from an elbow vein after an overnight fast (11–13 h). Samples were collected in the morning (07:00–09:00 h). Blood was collected in

Table 1. Baseline characteristics of the 28 study subjects

	Value
Gender	
Men (n)	14
Women (n)	14
Age (years)	36 \pm 11
Height (m)	1.74 \pm 0.1
Weight (kg)	70.1 \pm 13.8
BMI (kg m ⁻²)	23.1 \pm 3.2

Data are means \pm SD.

Table 2. Nutrient content in study juices

	Nutrient content per 100 ml juice	
	Grapefruit	Apple
Energy	45 kcal/180 kJ ^a	50 kcal/200 kJ ^a
Protein	0.5 g ^a	0.5 g ^a
Carbohydrates	8.8 g ^b	11.4 g ^b
Glucose	2.4 g ^b	2.7 g ^b
Fructose	2.3 g ^b	5.3 g ^b
Saccharose	2.5 g ^b	2.2 g ^b
Fat	<0.5 g ^a	<0.5 g ^a
Dietary fibre	0.4 g ^b	0.3 g ^b
β -Carotene	48 μ g ^b	0 μ g ^b
Retinol equivalents	8 μ g ^b	0 μ g ^b
Tocopherol	0.3 mg ^b	0 mg ^b
Ascorbic acid	24 mg ^a	0 mg ^c

^a From the juice packets.

^b From Swedish food-composition tables (14, 15).

^c From analysis performed by JO-bolaget (Stockholm, Sweden).

tubes, allowed to clot at room temperature for 1–2 h and centrifuged for 10 min at 3000 g. Serum was pipetted off into plastic vials and kept at -20°C . All samples of each individual were analysed in one batch after completion of the study to minimize analytical variation. All analyses were performed by accredited enzymic methods according to SS-ISO/IEC 17025 at the Central Laboratory for Clinical Chemistry at the Sahlgrenska University Hospital. LDL-cholesterol was calculated by Friedewald's formula as $\text{LDL} = \text{TC} - \text{HDL-cholesterol} - 0.45 \times \text{triglycerides}$.

Statistical analyses

Data are presented as mean values with standard deviation, standard error and ranges. The results between groups, as well as between periods and the interaction effect were analysed by Student's *t*-test, and within groups over time by paired *t*-test. Values were considered to be significantly different when $p < 0.05$. This study was designed to be able to detect a 16% decrease in TC, with a power of 90% and a significance level of 0.05. The design was based partly on previous results where grapefruit juice decreased total serum cholesterol by 16%, and partly on individual day-to-day variation and analytical variation in cholesterol of 7%.

Results

During the first period the body weight increased by 0.4 kg ($p = 0.02$). The subjects maintained their body weight during the washout and the second period. Overall, 98% of the prescribed volume of fruit juices was reported as consumed. Minor aberrations from the protocol were reported for 18 subjects. Nine subjects reduced their consumption of ordinary juice during the fruit juice periods. None of the subjects changed their consumption of

fruit and vegetables during the study. Eight, four and three subjects reported changes in their physical activity, diet or supplement habits, respectively.

Baseline serum values (mean and range) were: TC 5.0 (3.1 – 7.7) mmol l^{-1} , LDL-cholesterol 2.9 (1.2 – 5.5) mmol l^{-1} , HDL-cholesterol 1.6 (0.92 – 2.7) mmol l^{-1} and triglycerides 1.1 (0.49 – 2.5) mmol l^{-1} . HDL-cholesterol levels in women were higher ($p < 0.001$) and triglyceride levels were lower ($p < 0.005$) than in men.

There were no significant differences in the serum lipid responses between group A (starting with grapefruit juice) and group B (starting with apple juice); therefore, the results were combined for subsequent analyses.

Changes in serum lipid levels are summarized in Table 3. In the grapefruit juice periods, LDL-cholesterol decreased by 6% ($p = 0.04$). Apple juice did not significantly affect serum lipids, although there was a nominal decrease in LDL-cholesterol by 4% ($p = 0.27$). There was no significant difference in serum lipid levels after the periods with grapefruit juice and apple juice, respectively, and there were no significant differences in serum lipid responses between men and women or between subjects with elevated serum cholesterol ($\text{TC} > 5 \text{ mmol l}^{-1}$, $\text{LDL-cholesterol} > 3 \text{ mmol l}^{-1}$) and those with normal serum cholesterol ($\text{TC} < 5 \text{ mmol l}^{-1}$, $\text{LDL} < 3 \text{ mmol l}^{-1}$).

Throughout the 9 week study, TC and LDL-cholesterol decreased by 8% ($p < 0.0001$) and 14% ($p < 0.00001$), respectively, while HDL-cholesterol increased by 6% ($p = 0.028$) (Fig. 1). Serum triglyceride levels showed a tendency to decrease ($p = 0.12$). Even during the 3 week washout period, LDL-cholesterol decreased by 5% ($p = 0.036$). The serum responses of the subjects reporting changed

Table 3. Serum lipid levels (mmol l^{-1}) in 28 healthy adults during a 9 week cross-over trial with grapefruit juice and apple juice for 3 weeks interspaced by a 3 week washout period

Serum lipids	Grapefruit juice					Apple juice					Difference between juices	<i>p</i>
	Baseline	Week 3	Difference		<i>p</i>	Baseline	Week 3	Difference		<i>p</i>		
TC	4.88 ± 1.15	4.75 ± 1.17	-0.13 ± 0.44	–3%	0.13	4.88 ± 1.17	4.75 ± 1.14	-0.13 ± 0.62	–3%	0.30	0 ± 0.86	0.88
LDL	2.78 ± 1.04	2.61 ± 1.05	-0.17 ± 0.43	–6%	0.04	2.78 ± 1.05	2.68 ± 0.99	-0.10 ± 0.47	–4%	0.27	0.07 ± 0.73	0.61
HDL	1.64 ± 0.41	1.65 ± 0.18	0.01 ± 0.18	+1%	0.70	1.64 ± 0.49	1.67 ± 0.40	0.03 ± 0.26	+2%	0.58	0.01 ± 0.35	0.84
TG	1.04 ± 0.51	1.06 ± 0.57	0.02 ± 0.56	+2%	0.83	1.03 ± 0.39	0.93 ± 0.35	-0.10 ± 0.32	–10%	0.12	-0.12 ± 0.64	0.32

Data are means \pm SD.

TC: total serum cholesterol; LDL: low-density lipoprotein cholesterol; HDL: high-density lipoprotein cholesterol; TG: serum triglycerides.

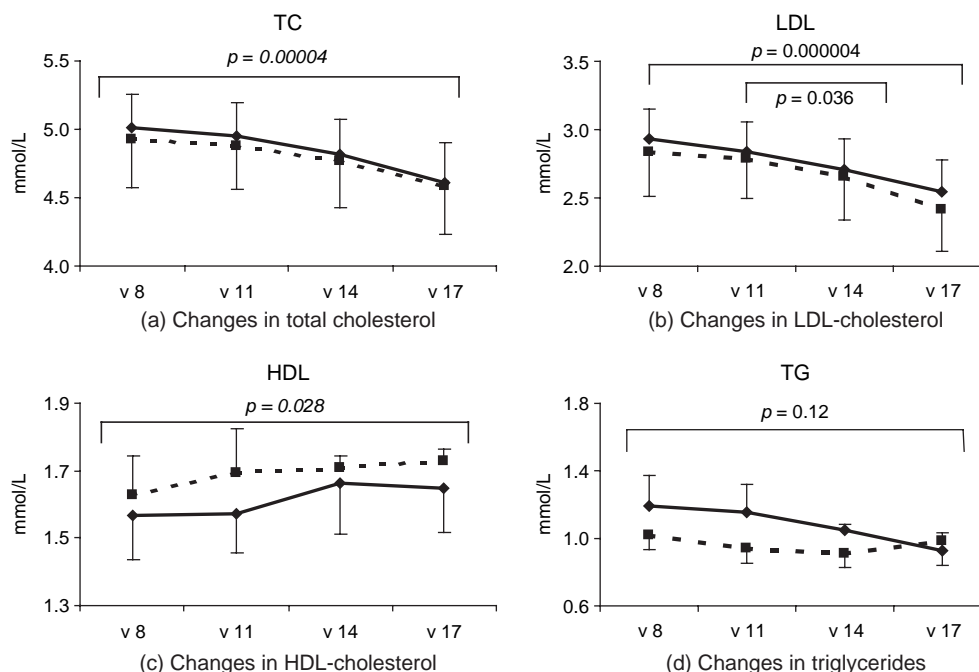


Fig. 1. Changes in serum lipids in 28 healthy adults during a 9 week cross-over trial with grapefruit juice and apple juice for 3 weeks interspaced by a 3 week washout period. (a) Changes in total cholesterol (TC); (b) changes in low-density lipoprotein (LDL)-cholesterol; (c) changes in high-density lipoprotein (HDL)-cholesterol; (d) changes in triglycerides (TG). Values are means with SE. Differences over time within groups evaluated by paired *t*-test. ■: Subjects on grapefruit juice between week 8 and 11, followed by 3 week washout, and apple juice between week 14 and 17 ($n = 13$); ◆: subjects on apple juice between week 8 and 11, followed by 3 week washout, and grapefruit juice between week 14 and 17 ($n = 15$).

habits in diet or physical activity were not different from the responses of other subjects.

Discussion

The present study shows that consumption of 250 ml grapefruit juice daily for 3 weeks significantly decreased serum LDL-cholesterol levels in healthy adults, whereas consumption of the same amount of apple juice did not. However, there was also a secular trend in LDL-cholesterol decrease in the apple juice periods and thus no statistically significant differences were found between the periods with grapefruit juice and apple juice. LDL-cholesterol also decreased during washout, further indicating that the effects of grapefruit juice per se on LDL-cholesterol are uncertain. The study showed a strong time effect in TC and LDL-cholesterol. During the 9 weeks of the study, there was a significant decrease in TC and LDL-cholesterol, whereas HDL-cholesterol levels increased. Therefore, the decrease in LDL-cholesterol with grapefruit juice cannot be separated from the time effect. Subgroup analysis revealed no differences in lipid response depending on gender or baseline cholesterol levels.

There are some limitations in this study to which attention should be paid. The intervention periods

were only 3 weeks, but dietary changes usually have full effects on serum lipids within a month (16), and many controlled cross-over studies have used periods of 3 weeks (17). This study used a cross-over design in which each subject received both active juice and control juice for 3 weeks. Thus, it seems improbable that it would have missed any potential lipid-lowering effect of grapefruit juice.

As the diet questionnaire was designed to detect dietary changes rather than absolute dietary intake, no information was obtained on the total energy intake of the subjects during the fruit juice and washout periods. Some subjects changed their dietary or physical activity habits during the study; however, the subjects who reported changes in their food, activity or supplement habits had similar effects on serum lipid levels as the other subjects. Underreporting or false reporting might have occurred, but no differences in this respect were seen between the periods. In addition, by using apple juice as placebo instead of water, the subjects were unaware which fruit juice was the active treatment.

By open recruitment of interested volunteers, who may have been more health conscious than the general population, these results could be biased. However, the dietary habits, physical activity habits

and original serum lipids levels found in this study were not different from those of the Swedish population in general (13).

Serum lipids show seasonal variation, with a peak in the winter and a trough in the summer (18, 19), but the actual decrease as demonstrated in this study was more accentuated than expected, and this was underlined by the significant decrease in LDL during the washout period. A possible explanation for this apparent general time effect could be the recruiting bias; subjects interested in dietary effects of fruit juice on cholesterol metabolism were more likely to volunteer for the study, and such subjects might be more inclined inadvertently to change their dietary and lifestyle habits when engaged in a study. However, with four regularly spaced occasions for serum samples this effect would have been as strong during both juice periods as during the washout. The small increase in body weight during the first period is probably due to the two subjects who reported increased dietary intake, because when they were excluded weight changes were no longer significant. There was no correlation between individual weight changes and serum lipid changes.

A previous open study (12) reported that Jaffa Sweetie juice reduced serum lipid levels by 16% in hypercholesterolaemic patients. However, there was no information on statistical power, compliance, or whether the patients changed their lifestyle regarding dietary and activity habits, or their serum lipids before the study.

It is possible but unproven that the presence of minor components in grapefruit juice or in Sweetie juice may be responsible for the lipid-lowering effects reported previously. Fruit and vegetables rich in antioxidants are regarded as beneficial for human health (2), but so far there is scarce evidence that antioxidants influence serum lipids (6, 7). Dietary fibre may contribute to the lipid-lowering effect, as the grapefruit juice contained a higher level of soluble fibre than the apple juice. However, in animals on cholesterol-free diets, grapefruit juice has not been found to decrease serum lipids (9, 10), nor does orange juice have any effects on lipid levels in humans (20). Thus, there is so far only one study from one laboratory reporting any significant cholesterol-reducing effects of grapefruit juice. During preparation of this manuscript another study from the same laboratory reported hypocholesterolaemic effects of the addition of either blond or red fresh grapefruit to patients with mixed hyperlipi-

daemia, while only red grapefruit had any effect on serum triglycerides (21). The nutrient content in the fruit was similar regarding dietary fibre, ascorbic acid, flavonoids and anthocyanins, while the antioxidant capacity in several tests was higher in the red than in the blond grapefruit. Naringin levels were also higher in red than in blond grapefruit, but as naringin is poorly soluble in water, other components may be of more importance. It must be remembered, however, that this was an open study where the control group received no substitute for grapefruit, and thus any hypolipidaemic effect could be affected by confounding factors.

To summarize, consumption of 250 ml grapefruit juice daily for 3 weeks decreased LDL-cholesterol levels by 6% compared with baseline values, but this was not significantly different compared with apple juice. In addition, LDL-cholesterol levels decreased during the washout period. Thus, the hypocholesterolaemic effects of grapefruit juice cannot be separated from a general time effect encountered in this study.

Acknowledgements

We are grateful to all participants, and for the expert technical assistance by Vibeke Malmros, Elisabeth Gramatkovski, Mitra Ravand and Annica Alklind at the laboratory of the Department of Clinical Nutrition, Sahlgrenska University Hospital, and Sahlgrenska Academy at Göteborg University, Göteborg, Sweden. We acknowledge the support by Gunilla Ekström, JO-bolaget, Stockholm, Sweden, in providing juices and cinema tickets.

References

1. Schaefer EJ. Lipoprotein, nutrition and heart disease. *Am J Clin Nutr* 2002; 75: 191–212.
2. Alexander J, Andersson JA, Aro A, Becker W, Fogelholm M, Lyhne N, et al. Integrating nutrition and physical activity. *Nordic Nutrition Recommendations 2004*. Report No. Nord 2004:13. Copenhagen: Nordic Council of Ministers; 2004.
3. Jenkins DJA, Wolever TMS, Rao A, Hegele RA, Mitchell SJ, Ransom TPP, et al. Effect on blood lipids of very high intakes of fiber in diets low in saturated fat and cholesterol. *N Engl J Med* 1993; 329: 21–6.
4. Gorinstein S, Cvikrova M, Machackova I, et al. Characterization of antioxidant compounds in Jaffa Sweeties and white grapefruits. *Food Chem* 2004; 84: 503–10.
5. Lichtenthaler R, Marx F. Total oxidant scavenging capacities of common European fruit and vegetable juices. *J Agric Food Chem* 2005; 53: 103–10.

6. Zino S, Skeaff M, Williams S, Mann J. Randomised controlled trial of effect of fruit and vegetable consumption on plasma concentrations of lipids and antioxidants. *BMJ* 1997; 314: 1787–91.
7. Heart Protection Study Collaborative Group. MRC/BHF heart protection study of antioxidant vitamin supplementation in 20 536 high-risk individuals: a randomised placebo-controlled trial. *Lancet* 2002;360: 23–33.
8. Sembries S, Dongowski G, Mehrländer K, Will F, Dietrich H. Dietary fiber-rich colloids from apple pomace extraction juices do not affect food intake and blood serum lipid levels, but enhance fecal excretion of steroids in rats. *J Nutr Biochem* 2004; 15: 296–302.
9. Gorinstein S, Leontowicz H, Leontowicz M, Krzeminski R, Gralak M, Martin-Belloso O, et al. Fresh Israel Jaffa Blond (Shamouti) orange and Israeli Jaffa Red Star Ruby (Sunrise) grapefruit juices affect plasma lipid metabolism and antioxidant capacity in rats fed added cholesterol. *J Agric Food Chem* 2004; 52: 4853–9.
10. Kurowska EM, Borradaile N, Meade M, Spence JD, Carroll KK. Cholesterol-lowering effects of dietary citrus juices and their flavonoids. Studies in rats, mice and rabbits [abstract]. *Atherosclerosis* 1997; 134: 330.
11. Davidson MH, Dugan LD, Stocki J, Dicklin MR, Maki KC, Coletta F, et al. A low-viscosity soluble-fiber fruit juice supplement fails to lower cholesterol in hypercholesterolemic men and women. *J Nutr* 1998; 128: 1927.
12. Gorinstein S, Caspi A, Libman I, Katrich E, Lerner TH, Trakhtenberg S. Fresh Israel Jaffa Sweetie juice consumption improves lipid metabolism and increases antioxidant capacity in hypercholesterolemic patients suffering from coronary artery disease: studies *in vitro* and in humans and positive changes in albumin and fibrinogen fractions. *J Agric Food Chem* 2004; 52: 5215–22.
13. Becker W. Riksmaten 1997–1998. Kostvanor och näringsintag i Sverige Metod- och resultatanalys. Uppsala: Livsmedelsverket; 2002.
14. Swedish National Food Administration. Kolhydrater. Uppsala: Livsmedelsverket; 1996.
15. Swedish National Food Administration. Food composition tables. Energy and nutrients 2002. 5th edn. Uppsala: Swedish National Food Administration; 2001.
16. Sandström B, Marckman P, Bindslev N. An eight-month controlled study of a low-fat high-fibre diet: effects on blood lipids and blood pressure in healthy young subjects. *Eur J Clin Nutr* 1992; 46: 95–109.
17. Clarke R, Frost C, Collins R, Appleby P, Peto R. Dietary lipids and blood cholesterol: quantitative meta-analysis of metabolic ward studies. *BMJ* 1997; 314: 112–7.
18. Ockene IS, Chiriboga DE, Stanek EJ, Harmatz MG, Nicolosi R, Saperia G, et al. Seasonal variation in serum cholesterol levels. Treatment implications and possible mechanisms. *Arch Intern Med* 2004; 164: 863–70.
19. Donahoo WT, Jensen DR, Shepard TY, Eckel RM. Seasonal variation in Lipoprotein lipase and plasma lipids in physically active, normal weight humans. *J Clin Endocrinol Metab* 2000; 85: 3065–8.
20. Kurowska EM, Spence JD, Jordan J. HDL-cholesterol-raising effect of orange juice in subjects with hypercholesterolemia. *Am J Clin Nutr* 2000; 72: 1095–100.
21. Gorinstein S, Caspi A, Libman I, Tzvi Lerner H, Huang D, Leontowicz H, et al. Red grapefruit positively influences serum triglyceride level in patients suffering from coronary atherosclerosis: studies *in vitro* and in humans. *J Agric Food Chem* 2006; 54: 1887–92.

Lars Ellegård

Department of Clinical Nutrition
 Sahlgrenska Academy at Göteborg University
 Box 459
 SE-405 30 Göteborg
 Sweden
 Tel: +46 31 7733725
 Fax: +46 31 829475
 E-mail: Lasse.Ellegard@nutrition.gu.se