

LycoRed as an alternative to hormone replacement therapy in lowering serum lipids and oxidative stress markers: A randomized controlled clinical trial

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Abstract

Aim: Menopause is a pro-atherogenic state with a sharp rise in the incidence of coronary artery disease. This pilot study was designed as an equivalence randomized clinical trial to explore the potential of LycoRed (containing 2000 µg lycopene) as an alternative to hormone replacement therapy (HRT) for the prevention of coronary artery disease in postmenopausal women.

Methods: Forty-one healthy postmenopausal women were randomly allocated to receive either continuous combined HRT ($n = 21$) or LycoRed ($n = 20$) for six months. Serum lipid profile, marker of lipid peroxidation (malondialdehyde), and the level of endogenous antioxidant (glutathione) were measured at the baseline, and 3 and 6 months after the intervention in both groups.

Results: At 6 months, HRT resulted in a significant decrease in total cholesterol (TC) level by 23.5%, low-density lipoproteins (LDL) by 19.6%, and an increase in high-density lipoproteins (HDL) by 38.9%. The LycoRed group showed similar changes in TC (–24.2%), LDL (–14.9%) and HDL (+26.1%). Triglyceride levels showed a smaller though significant increase at 6 months, but not at 3 months, in both groups. There was no significant change in the very LDL (VLDL) level in either group. Malondialdehyde levels decreased significantly by 16.3% and 13.3%, whereas glutathione levels increased significantly by 5.9% and 12.5% in HRT and LycoRed groups, respectively.

Conclusion: Both HRT and LycoRed had a favorable effect on serum lipids and oxidative stress markers which were comparable. LycoRed can be used as an alternative to HRT to reduce the risk of atherosclerosis in postmenopausal women.

Key words: hormone replacement therapy, lipids, LycoRed, oxidative stress, postmenopausal.

Introduction

Coronary artery disease (CAD) remains the leading cause of death in women, with its incidence rising sharply after menopause. The cardio-protective effect of estrogens is supported by the fact that bilateral oophorectomy in young women has been shown to increase the risk of CAD which can be eliminated by

estrogen replacement.¹ A meta-analysis of case control, cross-sectional and prospective studies observed a net protective effect of hormone replacement therapy (HRT) on coronary heart disease, particularly in current users.² However, the recent Women's Health Initiative randomized controlled trial reported an overall increased risk of CAD and a marginal increase in the risk of breast cancer in women on combined

Received: August 2 2005.

Accepted: February 13 2006.

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continuous hormone replacement therapy.³ This has resulted in a significant fall in compliance to HRT,⁴ and a renewed interest in alternative therapies for treating menopausal symptoms and prevention of postmenopausal coronary heart disease.

There is growing evidence that oxidative stress plays an important role in the pathogenesis of atherosclerosis because the low-density lipoproteins need to be oxidized to acquire atherogenic potential. Among the carotenoids studied for their antioxidant properties, lycopene derived from tomatoes and tomato based products, has been found to be the most efficient singlet oxygen quencher.⁵ Epidemiological studies have also demonstrated that the serum lycopene concentration has a significant inverse correlation with LDL oxidizability in healthy female volunteers,⁶ and higher levels of lycopene are associated with a lower risk of CAD in women.⁷

Therefore, this equivalence randomized clinical trial was designed to compare the effect of LycoRed and HRT in postmenopausal women on serum lipids, malondialdehyde (MDA) level, an intermediary marker of lipid peroxidation and glutathione (GSH), an endogenous antioxidant.

Materials and Methods

The study was conducted at the Department of Obstetrics and Gynecology, All India Institute of Medical Sciences, New Delhi from January 2002 to December 2003. Women attending the Menopause clinic were assessed for eligibility to enter the trial. The inclusion criteria was women less than 60 years of age with cessation of menses more than 1 year, or more than 6 months with FSH level $>40 \mu\text{L}$. Women with prior hysterectomy, smokers, and chronic medical disorders like diabetes, hypertension or heart disease were ineligible to participate in the study. Women taking lipid-lowering agents, antioxidants, or those who had ever used hormone replacement therapy were also excluded from the study. Written informed consent was obtained from all the study subjects, and the study was approved by the institutional ethics committee.

Seventy-two women were screened for eligibility to participate in the trial. A CONSORT flow diagram is shown in Figure 1. Forty-one women were randomly allocated into two groups using computer generated computer numbers. Women in group 1 ($n = 21$) received oral HRT (estradiol valerate 2 mg and norethisterone acetate 1 mg) and those in group 2 ($n = 20$) received LycoRed (2 softules each containing 2000 μg

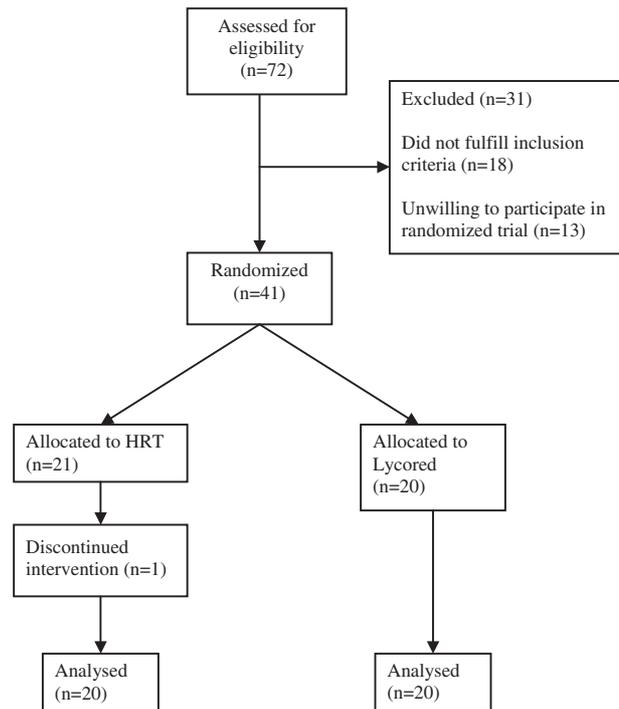


Figure 1 Flow diagram of the progress through the phases of a randomized trial.

of lycopene) daily for 6 months. Other constituents of LycoRed are vitamin A 2500 international units (IU), α -tocopheryl acetate 10 IU, vitamin C 50 mg, zinc sulfate monohydrate 27.45 mg, and monohydrated selenium dioxide 70 mg. One woman in group 1 refused to continue HRT after 1 month, although she did not complain of any significant side-effects. Therefore, she was excluded from the final analysis.

A detailed history, clinical examination and baseline investigations were performed to exclude significant medical illness. A complete serum lipid profile including total cholesterol (TC), high-density lipoproteins (HDL), low-density lipoproteins (LDL), very low-density lipoproteins (VLDL), and triglycerides (TG) were done at recruitment and repeated after 3 and 6 months of therapy in both arms of the study. Serum MDA and GSH levels were also measured at 0, 3, and 6 months in each group.

Laboratory methods

Five mL of venous blood was taken from each study subject and the serum separated and stored at -70°C until analysis for the estimation of MDA and GSH.

Estimation of malondialdehyde level

MDA estimation in serum was done by the method described by Ohkawa *et al.*⁸ To 0.1 mL of serum, 0.2 mL of 8.1% sodium dodecyl sulfate, 1.5 mL of 20% acetic acid and 1.5 mL of 0.8% thiobarbituric acid were added. The mixture was heated for 60 min at 95°C in temperature controlled water. After cooling 5 mL of *n*-butanol, pyridine (15:1) was added and vortexed. The mixture was centrifuged at 8000 g for 10 min and the upper organic layer was separated. Absorbance of the organic layer was read spectrofluorometrically at excitation wavelength 532 nm and emission wavelength 515 nm. 1,1,3,3-tetraethoxy propane was used as a standard to obtain a curve for the calculation of MDA in serum samples.

Estimation of reduced glutathione

GSH estimation was performed by the method described by Ellman *et al.*⁹ A protein free supernatant was obtained by the addition of equal volume of 10% trichloroacetic acid to the serum and centrifuged at 8000 g for 10 min. To 1 mL of this supernatant, 4 mL of 0.3M Na₂HPO₄ (pH 8.0) and 0.5 mL of 0.6 mmol/L, 5,5 dithiobis - 2 nitrobenzoic acid (DTNB) prepared in 1% trisodium citrate was added. The contents were vortexed and absorbance of yellow color produced, which was recorded within 10 min by a spectrophotometer (DU 640, Beckman, Fullerton, CA, USA) at 412 nm. A parallel standard of GSH was prepared to calculate the amount of GSH in serum sample.

Statistical analysis

Data were recorded on a predesigned performa and managed on an Excel spreadsheet. All entries were checked for any error. After checking for normality, lipid parameters and oxidative stress markers were summarized by arithmetic mean and standard deviation. Student's *t*-test was used to compare baseline lipid parameters and oxidative stress markers. To determine changes in lipid parameters and oxidative stress markers within each group, repeated measures ANOVA followed by Bonferroni post hoc analysis was used. Student's *t*-test was used to compare mean of lipid parameters and oxidative stress markers at baseline and at each follow-up visit. Baseline values for GSH were significantly different in the two groups; therefore, we used ANCOVA to compute the mean values of GSH at 3 months and 6 months, adjusting for imbalance in GSH at the baseline. We also computed percentage change in the various parameters from

baseline to 3 months and 6 months. The difference in the median percentage change between the two groups at 3 months and 6 months were compared using Wilcoxon rank sum test. STATA 8.0 & SPSS 10.1 statistical software was used for data analysis. In this study, $P < 0.05$ was considered as statistically significant.

Results

Women presenting to the Menopause clinic with menopausal symptoms or for a routine health check-up were included in the study. All the patients belonged to the middle income group. None of the patients suffered from any significant medical illness or were on any chronic medication. The mean age and BMI of women in the HRT group were 46.2 years and 25.3 in the HRT group and 46.4 years and 25.8 in the LycorRed group, respectively, which was comparable. The duration of menopause in both the groups was between 1 and 3 years, and there was no significant difference in the two groups.

Lipid profile

Table 1 shows the serum levels of TC, HDL, LDL, VLDL, and TG at baseline, 3 months and 6 months in the two groups. The TC level decreased significantly from 204.31 ± 22.1 mg/dL to 183.7 ± 31.9 mg/dL ($P = 0.001$) at 3 months and 148.5 ± 26.5 mg/dL ($P = 0.001$) at 6 months in the HRT group. The LycorRed group showed a similar reduction in TC from 215.1 ± 31.3 mg/dL to 189.2 ± 27.2 mg/dL ($P = 0.001$) and 161.5 ± 34.3 mg/dL ($P = 0.001$) at 3 and 6 months, respectively. The HDL levels increased significantly in both groups, the rise being more marked at 6 months in the HRT group ($P = 0.01$). Significant reduction occurred in LDL levels but no change in VLDL levels was observed in both the groups. Serum triglyceride levels showed no significant change from 0 to 3 months but increased significantly in both the groups at 6 months. The median percentage change in the lipid parameters at 3 and 6 months from the baseline is shown in Table 2.

Markers of oxidative stress

When the markers of oxidative stress were compared, MDA levels decreased in both groups significantly. The mean MDA level decreased from 37.7 ± 7.2 μ mol/L to 33.7 ± 5.4 μ mol/L ($P = 0.009$) and 31.8 ± 4.1 μ mol/L ($P = 0.001$) at 3 and 6 months in the HRT group and from 38.3 ± 6.3 μ mol/L to 34.7 ± 4.0 μ mol/L ($P = 0.026$) and 34.0 ± 3.7 μ mol/L ($P = 0.015$) in the LycorRed

Table 1 Serum lipid profile and oxidative stress markers in HRT and LycoRed groups

Parameter	Group	0 month Mean \pm SD	3 month Mean \pm SD	6 month Mean \pm SD	Repeated measures ANOVA		Post hoc analysis	
					F	P	0 versus 3 month	0 versus 6 month
TC (mg/dL)	HRT	204.3 \pm 22.1	183.7 \pm 31.9	148.5 \pm 26.5	54.80	0.0001	0.001	0.001
	Lyco	215.1 \pm 31.3	189.2 \pm 27.2	161.5 \pm 34.3	45.09	0.0001	0.001	0.001
HDL (mg/dL)	HRT	46.8 \pm 9.3	55.5 \pm 8.6	65.1 \pm 3.6	67.24	0.0001	0.001	0.001
	Lyco	47.6 \pm 1.9	53.7 \pm 7.8	59.3 \pm 7.4**	61.21	0.001	0.001	0.001
LDL (mg/dL)	HRT	131.8 \pm 18.6	119.2 \pm 16.5	105.2 \pm 11.8	56.10	0.0001	0.001	0.001
	Lyco	132.0 \pm 32.8	116.7 \pm 24.7	107.8 \pm 19.6	30.13	0.001	0.001	0.001
VLDL (mg/dL)	HRT	25.7 \pm 6.0	25.7 \pm 6.3	27.5 \pm 6.8	1.54	0.22	NS [†]	NS [†]
	Lyco	25.6 \pm 1.1	26 \pm 3.5	25.7 \pm 4.2	0.07	0.92	NS [†]	NS [†]
TG (mg/dL)	HRT	128.1 \pm 30.3	136.7 \pm 25.9	141.1 \pm 28.2	7.41	0.002	NS [†]	0.001
	Lyco	123.0 \pm 28.3	127.8 \pm 21.6	134.3 \pm 16.9	8.23	0.001	NS [†]	0.01
MDA (μ mol/L)	HRT	37.7 \pm 7.2	33.7 \pm 5.4	31.8 \pm 4.1	16.5	0.0001	0.009	0.001
	Lyco	38.3 \pm 6.3	34.7 \pm 4.0	34.0 \pm 3.7	7.93	0.001	0.026	0.015
GSH (μ mol/L)	HRT	8.7 \pm 1.5	9.2 \pm 1.2 [‡]	9.7 \pm 2.5 [‡]	8.26	0.001	0.003	0.036
	Lyco	7.5 \pm 1.2*	9.0 \pm 1.3 [‡]	9.8 \pm 1.9 [‡]	13.11	0.001	0.01	0.003

GSH, glutathione; HDL, high-density lipoproteins; HRT, hormone replacement therapy ($n = 20$), LDL, low-density lipoproteins; Lyco, LycoRed ($n = 20$); TC, total cholesterol; MDA, malondialdehyde; TG, triglycerides; VLDL, very low-density lipoproteins. Significant difference in mean levels between the two groups at 0 and 6 months * $P < 0.05$, ** $P < 0.01$. [†]No significant change in mean levels from 0–3 months and 0–6 months ($P > 0.05$). [‡]Mean values of GSH adjusted for imbalance at 0 month using analysis of covariance.

Table 2 Percentage median change in serum lipids and oxidative stress markers with HRT and LycoRed

Parameter	Group	0–3 month	0–6 months
		% Median change (range)	% Median change (range)
TC	HRT	-8.8 (-36.1, -0.8)	-23.5 (-50.8, -11.4)
	Lyco	-9.0 (-39.0, 0)	-24.2 (-52.0, -5.7)
HDL	HRT	14.9 (1.4, 56.5)	38.9 (4.2, 178.2)
	Lyco	14.8 (-1.4, 36.8)	26.1 (-1.4, 63.1)
LDL	HRT	-11.3 (-24.6, 13.2)	-19.6 (-31.5, -7.5)
	Lyco	-9.1 (-26.6, -1.8)	-14.9 (-38.6, 8.1)
VLDL	HRT	5.3 (-44.4, 39.1)	12.1 (-25.9, 73.9)
	Lyco	1.6 (-21.4, 25.0)	1.8 (24.1, 62.5)
TG	HRT	5.4 (-14.2, 54.5)	9.0 (-7.1, 44.5)
	Lyco	4.5 (-14.2, 25.0)	8.3 (-14.1, 41.0)
MDA	HRT	-10.8 (-30.0, 12.5)	-16.3 (-31.5, 9.0)
	Lyco	-8.6 (-28.5, 18.1)	-13.3 (-33.3, 25.0)
GSH	HRT	8.6 (-8.3, 40.0)	5.9 (-10.0, 40.0)
	Lyco	10.2 (-11.1, 40.7)	12.5 (-11.1, 55.6)

GSH, glutathione; HDL, high-density lipoproteins; HRT, hormone replacement therapy ($n = 20$), LDL, low-density lipoproteins; Lyco, LycoRed ($n = 20$); TC, total cholesterol; MDA, malondialdehyde; TG, triglycerides; VLDL, very low-density lipoproteins. Difference between the two treatment groups was statistically insignificant ($P > 0.05$) for all the parameters both at 0–3 months and 0–6 months.

group (Table 1). GSH levels showed a significant increase in both groups. The baseline GSH levels in the two groups were marginally but significantly different, although they were all within the normal range. However, to ensure that the post-intervention values in the two groups were compared to the pre-intervention values in a fair manner, an ANCOVA (regression analysis approach) was used by taking the baseline values of the variable as covariate for adjustment of post values. Therefore, the values of MDA at 3 and 6 months were

adjusted for this imbalance at the baseline. The MDA level decreased by 16.3% and 13.3% after 6 months, whereas GSH level increased by 5.9% and 12.5%, in HRT and LycoRed arms, respectively.

Discussion

Generation of free oxygen radicals or reactive oxygen species is a by-product of the essential oxidative processes in the body. These free radicals are unstable

chemical species with unpaired electrons capable of performing strong oxidation. They attack biologically important macromolecules like DNA, lipoproteins and carbohydrates, a mechanism which has been implicated in the pathogenesis of various diseases including CAD, respiratory diseases, rheumatoid arthritis, and cancer. To counteract this oxidative damage, the body has an efficient antioxidant system consisting of enzymes (glutathione peroxidase and reductase, superoxide dismutase), vitamins (C and E) and colored pigments (carotenoids). If this balance is disturbed, oxidative stress manifests resulting in disease. The oxidation of lipoproteins seems to be a key factor in the development of atherosclerotic plaques, because the lipid laden macrophages found in these plaques have few receptors for LDL in its original form but abundant receptors for oxidized LDL.¹⁰ Besides formation of the atherosclerotic plaques, the oxidized LDL and free oxygen radicals promote atherosclerosis by other pathways.^{10,11} Studies including men with CAD have shown a significant correlation between the susceptibility to LDL oxidation and activity of glutathione peroxidase 1 with severity of coronary atherosclerosis and cardiovascular events.^{10,12} Two other studies that looked at the correlation of serum lycopene with CAD and common carotid artery intima-media thickness (CCA-IMT) found an increased risk of acute coronary events and stroke and a significantly higher CCA-IMT, respectively, in subjects with lower lycopene levels.^{13,14}

Premenopausal women have a relatively lower incidence of ischemic heart disease as compared to men and postmenopausal women. This protection is believed to be because of the higher level of estrogens in pre-menopausal women.¹⁵ The proposed mechanisms of cardioprotection by estrogens include a favorable effect on the lipid profile, direct effect on vascular wall, and their antioxidant activity.¹⁶ Serum lipid peroxide levels have been reported to be lower in women than men in the age group of 30–50 years,¹⁷ and the level of antioxidant enzymes like glutathione peroxidase and superoxide dismutase are decreased in peri- and postmenopausal in comparison to premenopausal women.¹⁵

Even though there is ample evidence to implicate oxidative stress as one of the factors operational in the atherosclerotic process and development of CAD, the question that remains unanswered is whether its level can be brought down with therapeutic interventions and reduce the risk of CAD in men and women at risk. Epidemiological studies based on the evaluation of dietary intake of carotenoids and vitamins have shown

a significantly lower risk of CAD in women who consumed food rich in vitamin E,¹⁸ alpha-carotene or beta-carotene,¹⁹ a mixture of natural carotenoids²⁰ and who had a higher intake of lycopene from tomato-based products.⁷ These results however, have not been reproduced consistently in studies where antioxidant vitamins or β -carotene were administered as supplements instead of dietary content, with results reported both in favor and against a possible protective role in CAD.^{21–23} However, no published studies have investigated the effect of lycopene supplementation to reduce the risk of atherosclerosis and CAD in healthy postmenopausal women.

In regards to the effect on lipid profile, there is enormous data on the favorable effect of estrogens on serum lipids, but studies investigating the effect of carotenoids or lycopene on lipids are lacking. Very recently, Ahuja *et al.* reported a favorable effect of olive oil and tomato lycopene on the lipid profile in healthy individuals, although the mechanism was not clear.²⁴ Reduction in the serum TC and LDL has been reported in patients receiving unopposed or combined HRT, both by oral and parenteral route.^{25–28} However, the data on other lipids is less consistent with studies showing an increase, decrease or no change in HDL levels.^{25,27,29} Serum triglycerides have been found to be increased with oral and decreased with transdermal estrogen therapy.^{28,29}

In our present study, we observed a significant fall in TC and LDL, and an increase in HDL and TG in both the continuous combined HRT and the LycoRed group. The oxidative stress markers also showed a positive change, which was similar in both the groups. This could imply that in the present scenario, when the physicians are reluctant to prescribe and the women less inclined to take HRT, lycopene could be used as an alternative agent to reduce the risk of atherosclerosis and CAD in postmenopausal women. However, these findings need to be confirmed in a larger study.

Acknowledgments

The study was funded by the All India Institute of Medical Sciences Research Grant.

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