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Effect of Feeding *Lactobacillus acidophilus* V_3 on the Lipemic Status of Humans of Different Age Groups

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ABSTRACT

The effect of a probiotic strain, *Lactobacillus acidophilus* V_3, a human vaginal isolate, was studied on the lipemic status of 27 human subjects of varying age groups, namely, 20-40 years (A_1), 40-60 years (A_2) and 60-80 years (A_3). Acidophilus milk prepared using this culture was fed to all the subjects at the rate of 200 ml per day for 20 consecutive days. Their fasting blood samples were analysed for lipid profile six times: twice each before, during and after feeding acidophilus milk, keeping a gap of 10 days between two subsequent collections. Analysis of data indicated that toal cholesterol significantly ($P<0.05$) reduced in group A_1, while ratios of LDL/HDL and total / HDL cholesterol declined in group A_2. In A_3 group, no significant change was found in any of the parametric values. The feeding did not alter significantly the values of serum triglycerides, LDL cholesterol and VLDL cholesterol in any group of volunteers. The changes in lipid profile parameters followed either a linear or quadratic trend over the study period.

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INTRODUCTION

Selected species of lactobacilli and bifidobacteria constitute the autochthonous beneficial microflora in human and higher animals (Alm, 1991). Their use as probiotics and their role in intestinal therapy is well established in therapeutic medicine (Clements *et al.*, 1983, Fernandes and Shahani, 1989, Alm, 1991).

The role of dietary lactobacilli in cholesterol reduction is currently being debated by scientists world-wide as one of the therapeutic aspect. Several reports have shown their potential as hypocholesterolemic agents *in vitro* and *in vivo* (Hepner *et al.* 1979, Khedkar *et al.* 1993a, 1993b). However, some workers did not observe such an effect (Lin *et al.* 1989, Mc Namara *et al.* 1989). Such variations can be attributed to the strain of culture, lipemic status of subjects, exercise, diet, sex, alcoholism, smoking, age, etc.

The incidence of atherosclerotic heart diseases involving the build-up of fatty substances on the inside of artery wall is high in patients with hypercholesterolemia (Hepner *et al.* 1979, Mayes, 1988). Age is considered to be an irreversible risk factor governing the occurrence of coronary heart diseases (CHD). According to the National

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Health Examination Survey, ischemic heart disease is the leading cause of death in males after age 35 and in all persons after 45. For both sexes, there is more than fivefold increase in the average annual incidences of myocardial infarction between ages 40 and 60 (Bierman, 1991). Considering the importance of age factor in prevalence of coronary heart diseases, the present study was undertaken to evaluate the effect of feeding *Lactobacillus acidophilus* V_3 on the blood lipid profile of hyperlipemic and normolipemic subjects falling under different age groups.

MATERIALS AND METHODS

Culture

Lactobacillus acidophilus V_3, a human vaginal isolate was obtained from culture collection of our Department. The strain was maintained by weekly sub-culturing in sterile reconstituted skim milk tubes. Prior to its use in preparing acidophilus milk, it was activated by two daily transfers in reconstituted skim milk. In the earlier studies this strain has been found to yield good quality fermented milk (Khedkar, 1988) and possess special properties like bile deconjugation, bile tolerance, antibacterial activity against some pathogens and cholesterol assimilation *in vitro* (Ashar and Prajapati, 1998). Hence, this strain was selected for the experiment.

Preparation of acidophilus milk

Low fat milk (1.6% fat, 8.5% SNF) in batch size of 10-20 liters was heated to 120°C for 10 minutes, cooled, inoculated with the active culture of *Lactobacillus acidophilus* V3 and incubated at 37°C till setting (16-18 h). The curd was then broken, added with pineapple essence and lemon yellow color, homogenized and packed in 200 ml aliquots in sanitized polyethylene pouches. The product was stored at 2-8°C till use.

Experimental protocol

Twenty seven human subjects enrolled in the feeding trial were selected from either Hospital OPD's or the University campus. Majority of subjects were patients suffering from primary hypercholesterolemia or that secondary to hypertension, diabetes, hypothyroidism, etc. Their personal data regarding dietary habits and medical history were recorded in a separate proforma. No restriction was put on the normal dietary habits of participants. They were also allowed to take regular medicine prescribed by doctor for treating secondary ailments, if any. However, none of the volunteers were taking cholesterol reducing drugs. They were classified into three groups on the basis of age, viz., 20-40 years (A_1), 40-60 years (A_2) and 60-80 years (stat), which consisted of 8, 12 and 7 volunteers, respectively. The blood samples of each volunteer were collected by veinpuncture six times, i.e. 10 days before start of the experiment, on the day of starting feeding, two times during feeding and two times after stopage of feeding. All the samples were analysed for lipid profile parameters and the data obtained were statistically analysed.

Lipid profile analysis

Total cholesterol, serum triglycerides and HDL-cholesterol were estimated with the help of commercial kits manufactured by M/S Bayer Diagnostics India Ltd., Baroda as per the methods suggested by them. The values of VLDL cholesterol, LDL cholesterol, LDL/HDL cholesterol ratio and total/HDL cholesterol ratio were derived from result of the former three parameters.

Total cholesterol estimation

Total cholesterol was estimated by an enzymatic method according to the procedure given in reagent kit manual (M/S Bayer Diagnostics India Ltd., Baroda, India). Daily working solution was prepared by mixing equal volumes of solution (1) i.e. buffer / enzyme / chromogen and reagent (2) i.e. phenol, supplied in the kit. To one ml of this working reagent, 10 µl of test serum sample was added. Blanks and standards were also run along with the test. Subsequently, the tubes were incubated at 37°C in a waterbath for five minutes. Thereafter, the absorbance of test as well as the standards were read on Hitachi 220S spectrophotometer at 505 nm against a reagent blank. The concentration of total serum cholesterol was expressed in mg/dL.

Triglycerides determination

Serum triglycerides were estimated by enzymatic Dichloro-2-Hydroxy Benzene Sulfuric acid (DHBS) colorimetric method given in the reagent kit manual (M/S Bayer Diagnostics India Ltd., Baroda, India). The reagent I containing enzyme/chromogen was reconstituted by adding equal volumes of reagent 2 (buffer/chromogen) and reagent 3 (stabilizer) in the required quantity. This reconstituted solution was used as working reagent. One ml of this working reagent was taken in a test tube to which 10 µl of serum sample was added. Incubation of the tubes was carried out for 15 minutes at room temperature. Finally, the OD of standard as well as test was measured against reagent blank at 505 nm. The concentration of serum triglycerides was expressed in mg/dL.

HDL cholesterol estimation

HDL cholesterol was estimated by phosphotungstate method as given in the kit manual (M/S Bayer Diagnostics India Ltd., Baroda, India). Chylomicrons, VLDL and LDL fractions in serum were separated from HDL by precipitating with phosphotungstic acid-magnesium chloride. In this step, 200 µl of serum and 200 µl of precipitating reagent (phosphotungstic acid) were centrifuged for 10 minutes at 3500-4000 RPM. The clear supernatant obtained thus was taken as sample. The remaining procedure was similar to estimation of total cholesterol except that instead of 10 µl serum, 20 µl supernatant obtained thus was taken in one ml of working reagent. The concentration of HDL - cholesterol was calculated and expressed in mg/dL of sample.

The other parameters were calculated as follows :

VLDL cholesterol = Triglycerides / 5,

LDL cholesterol = Total cholesterol - HDL cholesterol - VLDL cholesterol

LDL / HDL cholesterol ratio = LDL cholesterol / HDL cholesterol

Total / HDL cholesterol ratio = Total cholesterol / HDL cholesterol

Statistical analyses

Individual grouped and pooled data were analyzed by Randomized Block Design for analysis of variance by taking test periods (days) as treatments and individuals as replications. The baseline values used for the analysis purpose were the average of two observations taken 10 days prior to feeding (- 10d) and on the day of starting feeding (0d). The significant differences between treatment means for respective parameters were tested by the Critical Difference (CD) test. Regression analysis was also done for individual groups to statistically assess the trend of lipid profile parameters. Linear equation $y = a + bx$ and quadratic equation $y = a + bx + cx^2$ were applied as per the method suggested by Snedecor and Cochran (1967). The best fit equation was selected on the basis of significance of value of coefficient of determination (R^2) to explain the trend of lipid profile parameters over time.

RESULTS AND DISCUSSION

Analyses of fresh acidophilus milk revealed that it has 1.6 % fat, 3.5 % protein, 3.6×10^9 viable cells of lactobacilli / ml and 0.66 % titratable acidity. Speck (1976) suggested to consume about 10^8-10^9 live cells of lactobacilli daily to get maximum therapeutic benefits. Accordingly, a daily dose of 200 ml of our fresh acidophilus milk was able to provide about 10^{11} viable cells which are sufficient to give therapeutic benefits.

The average values of lipid profile parameters in the volunteers belonging to groups A_1, A_2 and A_3 are given in Table - 1. Analysis of the data revealed a significant ($P<0.05$) reduction of total cholesterol in group A_1. The average total cholesterol reduced maximally by 25 mg/dL during twenty days after stoppage of acidophilus milk consumption. In the A_2 group, a significant reduction ($P<0.05$) in the LDL/HDL and total / HDL cholesterol ratios as well as a non-significant decrease in total cholesterol was found. These favourable changes were, however, accompanied by a non-significant rise in the average serum trigycerides and VLDL cholesterol levels. HDL cholesterol remained unchanged in A_1 and A_3 groups but had shown erratic behaviour in A_2. In the A_3 group, no significant change was found with respect to any of the parameters during study.

Table - 2 indicates the composite results of RBD and regression analyses carried out on the grouped data to detect the trend of lipid profile parameters over the test periods. Linear as well as quadratic trends were fitted taking all the seven parameters as dependent variables and the test periods as independent variables. The significance of the value of coefficient of determination (R^2) was kept as a criterion for selection of the best fit linear or quadratic trend. A high R^2 value above 0.7 indicates a good correlation between two variables. Table - 3 indicates the trend equations for the groups of volunteers showing R^2 value of more than 0.7. With respect to the total cholesterol, a non-significant quadratic trend of final decline was seen in A_1 as well as A_2 groups. In the A_3 group, neither linear nor quadratic trend fitted with respect to total cholesterol indicting a poor correlation with test period. The triglycerides and VLDL cholesterol values showed a significant quadratic trend of final

increase in the A_2 group. In the A_3 group, a significant linear trend of increase in the averages of triglycerids as well as quadratic trend of final increase in VLDL cholesterol values was found (Table - 3). The HDL cholesterol values of A_1 group showed a quadratic trend decreasing initially followed by subsequent rise. A significant quadratic trend ($P<0.05$) of final decline in average LDL cholesterol level was seen in A_1 group. In the A_2 group, a linear significant trend of continuous decline was detected, whereas the A_3 group showed a non-significant trend of quadratic decline in LDL cholesterol levels. With respect to the cholestrol ratios, the A_1 group exhibited a quadratic trend of initial increase followed by a final decline in the average LDL/HDL and total / HDL cholesterol ratios. However, in groups A2 and A3 the ratios did not show any specific trend.

The results indicated that the feeding of acidophilus milk made favourable changes in lipemic profile of volunteers of group A_1 (20-40 years) and A_2 (40-60 years). The A_3 group, consisting of 60-80 years aged individuals showed no significant change in the lipemic status probably because their initial cholesterol was low. Amongst the three age groups, A_2 showed a change with respect to every parameter of lipid profile (Table - 2) and was maximally affected by *Lb. acidophilus* feeding.

Ratios of LDL / HDL cholesterol and total / HDL cholesterol are indicative of the risk of developing coronary heart disease (Kaplan *et al.* 1988). Both these ratios were found to decrease significantly in the age group A_2 in the present study. This is an important observation as the incidence of coronary heart diseases, too, is the most pronounced during the age of 40-60 years. Khedkar *et al.* (1993b) found a significant reduction in the serum total cholesterol of 20 male volunteers aged 40 to 50 years on consumption of acidophilus milk in the dose of 200 g/day for 30 days. In a similar study by the same researchers (Khedkar *et al.* 1993a) taking 20 healthy human volunteers aged 50-60 years, too, a significant reduction of total cholesterol levels was found. They noted that the initial baseline mean value (226.5 mg/dL) reduced on days 15 and 30 during feeding (213.1 and 192.4 mg/dL, respectively) and remained almost same after 15 days post feeding (193.2 mg/dL).

Agerbek *et al.* (1995) have also reported a significant reduction in total cholesterol levels after 6 weeks daily feeding of 200 ml of cultured milk *Gaio* in 58 Danish male participants of 44 years age. Hruby *et al.* (1992) have reported decrease in total cholesterol and low density lipo proteins accompanied by a moderate increase in the HDL fraction and frequently a decrease in triglycerides on feeding two different low fat fermented milks to 25 women and 18 men lying in the age group of 26-60 years.

In A2 group, both the cholesterol ratios showed lowest values at the end of feeding and maintained low during post feeding period too. The significant decline in average total cholesterol level of A_1 group was observed in the post feeding periods of + 10 and + 20 days after stoppage of acidophilus milk consumption, indicating a gradual effect. To check how long this favourable effect persists, perhaps, it would be necessary to include one or two additional post feeding test periods in future studies.

TABLE 1

Average lipid profile parameters in test subjects over the study period.

Parameter	Group	Baseline	Feeding		Post feeding		S.Em.	C.D.	C.V. %
		0 day*	10 d	20 d	+10 d	+ 20 d			
Total	A_1	213	217	219	198	188	7.8	22.6	10.7
Cholesterol	A_2	215	198	197	198	194	8.2	NA	14.3
(mg/dL)	A_3	190	191	190	183	200	8.1	NA	11.2
Serum	A_1	160	154	152	135	166	19.7	NA	36.3
Trigiycerides	A_2	166	153	155	171	194	12.3	NA	25.4
(mg/dL)	A_3	122	140	143	150	158	21.1	NA	22.4
HDL	A_1	54	52	48	47	50	4.7	NA	26.8
Cholesterol	A_2	44	38	53	46	45	3.3	9.4	25.3
(mg/dL)	A_3	39	41	43	31	43	3.9	NA	26.2
VLDL	A_1	32	31	30	27	33	3.9	NA	36.3
Cholesterol	A_2	33	30	31	34	39	2.5	NA	25.4
(mg/dL)	A_3	24	28	29	30	31	2.4	NA	22.4
LDL	A_1	127	135	140	125	105	9.9	NA	22.1
Cholesterol	A_2	138	129	112	117	109	8.6	NA	24.5
(mg/dL)	A_3	126	122	119	123	126	8.6	NA	18.5
LDL/HDL	A_1	2.3	2.6	2.9	2.6	2.1	0.12	NA	20.9
ratio **	A_2	3.1	3.4	2.1	2.5	2.4	0.1	0.28	20.3
	A_3	3.2	3.0	2.8	4.0	2.9	0.1	NA	20.9
Total / HDL	A_1	4.0	4.2	4.6	4.2	3.8	0.1	NA	14.2
ratio **	A_2	4.9	5.2	3.7	4.3	4.3	0.08	0.24	13.3
	A_3	4.9	4.6	4.4	5.9	4.6	0.10	NA	16.3

* Average of two baseline estimations i.e. 10 days before feeding and on zero day.

** The orginal values given above were transformed to square root for statistical analysis.

NS : Non significant, A_1 : 20-40 years, A_2 : 40-60 years, A_3 : 60-80 years.

TABLE 2

Maximum decrease / increase in lipid profile parameters during the study.

	Group	Change in lipid profile parameters as compared to base line values						
		Total cholesterol (mg/dL)	Triglycerides (mg/dL)	HDL cholesterol (mg/dL)	VLDL cholesterol (mg/dL)	LDL cholesterol (mg/dL)	LDL/HDL ratio	Total/HDL ratio
n = 8	A_1	↓25.0*	=	↓7.0	=	↓22.0•	↓0.2•	↓0.2•
n = 12	A_2	↓21	↑28.0•	↓6.0*	↑6.0•	↓29•	↓1.0•	↓1.2*
n = 7	A_3	↑10.0	↑36.0•	=	↑7.0•	↓7.0	=	=

A_1 : 20-40 years, A_2 : 40-60 years, A_3 : 60-80 years.

* : Significant as 5% by R.B.D., • : Significant at 5% by regression analysis

= : No change, ↓ : Decrease, ↑ : Increase

TABLE 3

Best fit trend equations for lipid profile parameters in different age groups.

Parameter	*Group*	*Constant (a)*	*Rate of Change*		*Coefficient of Determination*	*Significance at 5 % level*
			(b1)	*(b2)*	*(R^2)*	
Total	A_1	203	14.70	-3.60	0.93	NS
Cholesterol	A_2	227	-16.20	2.00	0.84	NS
Triglycerides	A_2	192	-31.00	6.30	0.98	*
	A_3	118	8.20	0.00	0.93	*
HDL Cholesterol	A_1	61	-7.00	0.90	0.91	NS
VLDL	A_2	38	-6.10	1.30	0.99	*
Cholesterol	A_3	21	4.20	-0.40	0.96	*
LDL	A_1	106	26.10	-5.30	0.98	*
Cholesterol	A_2	142	-7.00	0.00	0.82	*
	A_3	133	-8.90	1.50	0.91	NS
LDL/HDL	A_1	1.7	1.00	-0.20	0.97	*
TC/HDL	A_1	3.2	1.10	-0.20	0.91	NS

A_1 : 20-40 years, A_2 : 40-60 years, A_3 : 60-80 years

NA : Not Significant, * : Significant at 5 % level

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