Indian J Physiol Pharmacol 2005; 49 (1): 49–56

SERUM LIPID RESPONSE TO INTRODUCING GHEE AS A PARTIAL REPLACEMENT FOR MUSTARD OIL IN THE DIET OF HEALTHY YOUNG INDIANS

S. RAVI SHANKAR, R. K. YADAV, ROOMA BASU RAY, R. L. BIJLANI†, TARUN BAVEJA*, NISHI JAUHAR*, NIRANKAR AGARWAL*, SUMAN VASHISHT**, S. C. MAHAPATRA, NALIN MEHTA AND S. C. MANCHANDA**

*Departments of Physiology and **Cardiology, All India Institute of Medical Sciences, New Delhi – 110 029*

**The Mother's Health Centre, Sri Aurobindo Ashram (Delhi Branch), New Delhi – 110 016*

(Received on July 17, 2004)

Abstract : Ghee (clarified butter) has generally been assumed to be hypercholesterolaemic on the basis of its composition but there is hardly any study to support or refute the assumption. The present study was conducted on sixty-three healthy, young, physically active adult volunteers (52 male, 11 female). The study design was that of a randomized controlled trial with a parallel design. After a lead-in period of 2 wk, the subjects were randomly divided into two groups, Group A (n = 30; 25 male, 5 female) and Group B (n = 33; 27 male, 6 female). Group A (experimental) consumed for 8 wk a diet in which ghee provided 10% of the energy intake. The only other visible fat in the diet was mustard oil, and total energy from fats was 25% of the energy intake. Group B (control) consumed for 8 wk a similar diet except that all visible fat came from mustard oil.

The serum total cholesterol level showed a significant rise in the experimental group at 4 wk; the rise persisted at 8 wk. A similar rise was also seen in HDL cholesterol. Hence the total cholesterol/HDL cholesterol ratio did not show any significant change. In the control group, there was a trend towards a fall in LDL cholesterol but the change was not significant. The study does not indicate any adverse effect of ghee on lipoprotein profile. However, more studies are needed on older subjects, hyperlipidaemic subjects, and on subjects following less healthy lifestyles before the results of this study can be extrapolated to the general population.

Key words : ghee (clarified butter), fats, dietary fats, cholesterol, serum lipids, lipoproteins, coronary heart disease, atherosclerosis

†Corresponding Author : (E-Mail : rambij@hotmail.com)

INTRODUCTION

India produces about 800,000 tonnes of ghee annually, much of it by the traditional method (1) of heating butter at a temperature of 110°C or a little higher until it contains less than 1% moisture, and the milk solids separate out and turn brown (2). The milk solids are separated by straining and discarded. The clear fat that remains is ghee. The impact of various dietary fats on risk for athersclerosis has been studied extensively during the last 4 decades, using serum lipoproteins as biochemical markers, which has led to several meta-analyses (3–5). But these studies have almost entirely been on dietary fats commonly used in western countries. However, that has not prevented ghee from becoming the subject of a poorly informed debate. Jacobson hypothesized that cholesterol oxides in ghee were possibly responsible for immigrant Indians being at a higher risk for atherosclerosis as compared to the native populations of western countries (6). Further, he calculated on the basis of data from animal studies that the atherogenic dose of ghee is likely to be only 1 g ghee per day. Jacobson's hypothesis invited some comments which speculated on several factors other than ghee, and the cholesterol oxides it contains, which could possibly contribute to atherosclerosis in Indians (7, 8). Aneja and Murthy (1) analysed ghee and found in it a relatively high content of conjugated linolenic acid (CLA), which has been reported to have anti-carcinogenic, anti-atherosclerotic and immuno-enhancing properties (9). Aneja and Murthy's article, in turn, invited a sharp comment which questioned whether its CLA content could comprensate for its high saturated fat content (10). While these debates have been largely speculative, medical opinion has generally been unfavourable to ghee (11) on the basis of its high saturated fat content. The bias against ghee ignores a few important facts. First, the positive correlations between saturated fat intake and cardiovascular mortality found in earlier studies have been absent, or even reversed, in larger, more recent studies (12). Second, the National Cholesterol Education Program of the U.S.A. permits about one-third of the fat intake in the form of saturated fats (13). Third, while ghee has 65% saturated fatty acids, it also has 32% monounsaturated fatty acids (MUFA) (14), and MUFA are hypocholesterolaemic (15). Fourth, cardiovascular morbidity and mortality has increased in India after the consumption of ghee has fallen sharply due to a steep rise in the price of ghee (7). Finally, there has hardly been any scientific study on the effects of ghee on coronary risk factors. In a study on 7 subjects done at the National Institute of Nutrition, when the subjects shifted from a diet providing 50 g of visible fat per day in the form of groundnut oil to one providing it in the form of 35 g ghee and 15 g groundnut oil for 8 wk, the mean levels of serum cholesterol and triglycerides were not altered, but in two subjects serum total cholesterol increased by more than 20 mg/dL (16). The study was thus inconclusive. From our previous preliminary study, it was tentatively concluded that consuming ghee at the level of 10% of the energy intake in a vegetarian diet has no significant effect on the serum lipid profile of young, healthy, physically active individuals (17). The present report is based on continuation of the study on additional subjects, which has

permitted more firm conclusions.

METHODS

Subjects

The study was conducted on 63 healthy, young, physically active adult volunteers (52 male and 11 female), all of them inmates of Sri Aurobindo Ashram, New Delhi. None of the subjects smoked or used alcohol. All the subjects had all their meals at a common kitchen in the ashram. The meals were vegetarian, and the principal cooking medium was mustard oil.

Experimental design

The study was conducted in the form of a randomized controlled trial with a parallel design. Although the study was conducted in two phases (Feb-Apr 2001 and Jan-Mar 2003), the design of the study was exactly the same in both phases. The study started with a lead-in period of 2 wk, during which the subjects were requested to consume a relatively constant vegetarian diet, take no ghee at all, avoid baked foods (because they may contain ghee), maintain a relatively constant level of physical activity, and make no changes in the cooking medium.

After the lead-in period of 2 wk, the subjects were randomly divided into two groups, Group A (n=30; 25 male, 5 female) and Group B (n=33; 27 male, 6 female). Group A (experimental) consumed for 8 wk a diet in which ghee provided 10% of the energy intake while total energy from fats was 25% of the energy intake. Group B (control) consumed for 8 wk a similar diet except that all visible fat came from mustard oil as during the lead-in period and, by and large, even before that (Fig. 1).

Ghee was provided to Group A in one dish (potato curry) such that the subject would get 5% of the day's energy intake from the visible fat in the dish at lunch, and 5% at dinner. Group B also received the potato curry, but for them it was prepared in mustard oil. The investigator who analyzed the blood samples did not know to which group a given subject belonged.

Fasting blood samples were collected at the beginning of the lead-in period (–2 wk), the end of the lead-in period (0 wk), at 4 wk and at 8 wk.

The Ethics Committee of the All India Institute of Medical Sciences approved the protocol of the study, and the volunteers gave their informed written consent for participation in the study.

Measurements

The measurements included total cholesterol, total triglycerides, HDL cholesterol and LDL cholesterol, which were estimated using kits from Randox Laboratories Ltd., Ardmore, U.K. Briefly, cholesterol or triglycerides in the sample were oxidised enzymatically releasing hydrogen peroxide. Hydrogen peroxide, in the presence of peroxidase, interacts with 4-chlorophenol and 4-aminoantipyrene to yield quinoneimine, which can be measured colorimetrically from absorbance at 546 nm. HDL cholesterol was measured after precipitating LDL and VLDL by addition of phosphotungstic acid in the presence of magnesium ions. LDL was

measured after precipitating it with heparin at pH 5.04.

The intra-assay and inter-assay variation was less than 5% for total cholesterol, HDL cholesterol and triglycerides. For LDL cholesterol, the intra-assay variation was less than 5% but the inter-assay variation was less than 10%.

During phase 1 of the study, serum Lp(a) was also measured at 0 wk and 8 wk; and during phase 2, serum malondialdehyde (MDA) was also measured in blood samples. Lp(a) was estimated using a kit from Randox Laboratories Ltd., Ardmore, UK. MDA was estimated by the method of Chaturvedi et al (18).

Statistical analysis

The statistical analysis was done using SYSTAT version 9.0 and epiinfo version 6.04 D. Repeated measure analysis was applied to examine the significance, if any, of changes over a period of time i.e. from 0 wk to 8 wk. In case of statistical significance, Neuman Keul's multiple range test was applied to evaluate the sets of observations which were significantly different from each other. Differences were considered significant if P was less than 0.05.

RESULTS

The physical characteristics of the subjects are shown in Table I. There were no significant differences between the two groups. Further, there was no significant change in body weight or body mass index in either group during the study. Analysis of the average daily diet on a typical day revealed that in the experimental group fat energy% was 24.2% and the PUFA: SFA ratio 0.71, and the corresponding values in the control group were 20.7% and 1.16.

TABLE I: Characteristics of the subjects.

	Group A (Experimental)	*Group B (Control)*
Age (years)	23.2±4.27	23.45±6.05
Weight (kg)		
At 0 wk	54.38±9.31	54.22±6.33
At 8 wk	54.58±9.15	53.81±6.33
Height (cm)	162.9±7.95	163.57±5.89
BMI (kg/m)		
At 0 wk	20.4±2.7	20.22±1.71
At 8 wk	20.35±2.68	20.06±1.62

All values are mean ± standard deviation.

The serum lipoprotein profiles at different points during the study in the experimental group and control group have been shown in Tables II and III. The total cholesterol level showed a marginal but significant rise in the experimental group at 4 wk; the rise persisted at 8 wk. Since a similar rise was also seen in HDL cholesterol, the total cholesterol/HDL cholesterol ratio did not show any significant change (Table II). The salient features of the results have been highlighted in Fig. 2.

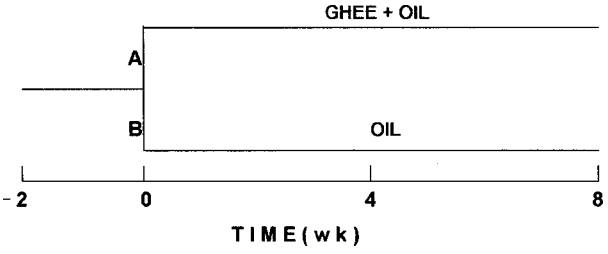

Fig. 1: The experimental design.

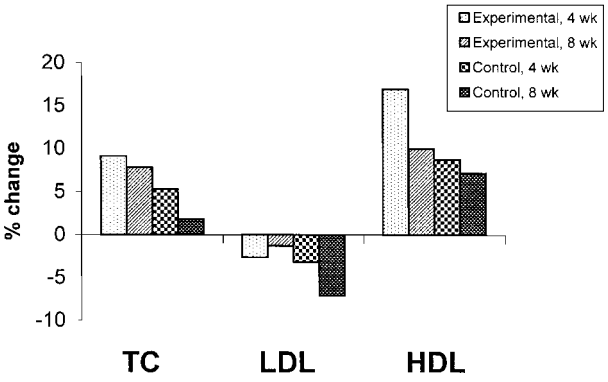

Fig. 2: Percentage change in lipoprotein profile in the experimental and control groups at 4 wk and 8 wk as compared to 0 wk. TC, total cholesterol; LDL, low density lipoprotein cholesterol; HDL, high density lipoprotein cholesterol.

Scrutiny of the raw data revealed considerable individual variation. The data of individuals who showed a greater than 15% rise in total cholesterol were analysed separately (Table IV). There were 8 such individuals in the experimental group, and 5 in the control group. Analysis of these subgroups revealed that the rise in cholesterol levels in the experimental group was primarily due to a rise in HDL levels, whereas in the control group, it was primarily due to a rise in LDL. That is why there was a significant rise in the total cholesterol/HDL cholesterol ratio in the control group but no change

TABLE II: Serum lipid profile in the experimental (ghee) group.

Variable	*Value (mean ± SD)*			
	–2 wk	*0 wk*	*4 wk*	*8 wk*
Total cholesterol (mg/dL)	163.98±34.65	155.28±29.99	169.39±41.68*	167.33±32.56*
LDL cholesterol (mg/dL)	97.38±26.11	95.93±22.71	93.43±24.61	94.68±23.83
HDL cholesterol (mg/dL)	49.37±9.73	42.89±5.71	50.07±13.48*	47.17±9.21*
Total cholesterol/HDL cholesterol ratio	3.38±0.72	3.63±0.59	3.47±0.79	3.65±0.83
VLDL cholesterol (mg/dL)	17.24±9.10	16.31±10.34	26.91±21.28	27.31±20.09*
Triglycerides (mg/dL)	85.85±24.78	89.09±30.09	105.64±47.90*	83.78±22.82

*P<0.05 (as compared to the 0 wk value)

TABLE III: Serum lipid profile in the control group.

Variable	*Value (mean ± SD)*			
	–2 wk	*0 wk*	*4 wk*	*8 wk*
Total cholesterol (mg/dL)	156.55±37.37	150.96±32.56	158.94±36.54	153.68±33.41
LDL cholesterol (mg/dL)	96.70±25.97	93.09±24.21	90.13±30.19	86.51±27.60
HDL cholesterol (mg/dL)	41.87±6.26	40.47±6.34	44.00±6.52	43.36±7.88
Total cholesterol/HDL cholesterol ratio	3.76±0.85	3.80±0.89	3.69±1.00	3.66±0.99
VLDL cholesterol (mg/dL)	18.67±13.84	18.13±12.57	25.70±17.89*	23.78±17.31*
Triglycerides (mg/dL)	93.80±34.34	86.29±38.91	93.48±36.99	77.35±23.85

*P<0.05 (as compared to the 0 wk value)

TABLE IV: Serum lipid profile in the subgroups with hypercholesterolemic response at 4 wk.

Variable	*Experimental group (n = 8)*			*Control group (n = 5)*		
	0 wk	*4 wk*	*8 wk*	*0 wk*	*4 wk*	*8 wk*
Total cholesterol (mg/dL)	15.9±39.6	197.4±52.2*	171.5±38.7	136.1±27.8	175.0±46.4*	149.5±23.4
LDL cholesterol (mg/dL)	99.9±32.1	101.1±40.3	106.9±35.6	79.9±34.8	101.0±54.3	81.2±31.3
HDL cholesterol (mg/dL)	44.6±6.5	65.5±16.4*	51.7±12.7	44.3±13.1	44.6±5.8	49.3±12.1
Total cholesterol/HDL cholesterol	3.58±0.71	3.12±0.98	3.45±0.85	3.30±1.18	4.02±1.30*	3.18±0.88

All values are expressed as Mean ± S.D.
*P<0.05 (as compared to the 0 wk value)

TABLE V: Lp(a) and MDA levels in the experimental and control groups.

Variable	*Experimental (ghee) group*			*Control group*		
	0 wk	*4 wk*	*8 wk*	*0 wk*	*4 wk*	*8 wk*
Lp(a) (mg/dL)	38.15±19.69	–	42.07±20.81	25.13±15.02	–	32.41±15.19
MDA (nmol/mL)	1.028±0.323	0.932±0.413	0.894±0.447	1.052±0.466	0.992±0.405	0.838±0.404*

All values are expressed as mean ± S.D.
*P<0.05 (as compared to the 0 wk value)

in this ratio in the experimental group (Table IV).

The limited data available on Lp(a) and MDA levels has been given in Table V. There was a trend towards a fall in MDA levels in both the groups, but the fall was significant only in the control group at 8 wk.

DISCUSSION

The results of the present study indicate that if ghee replaces mustard oil such that it contributes about 10% of the energy intake in the diet of healthy, young, physically active subjects on a vegetarian diet, there is a significant rise in serum cholesterol by 4 wk, and the rise persists till at least 8 wk. However, the rise in total cholesterol is primarily due to a rise in HDL cholesterol. Hence there is no significant change in total cholesterol/HDL cholesterol ratio. There is also a significant rise in serum triglycerides at 4 wk, but the levels return to the baseline by 8 wk (Table II). The rise in HDL cholesterol may be due to the considerable MUFA content of ghee, as observed also in case of palm oil (19). In the control group, there is no significant change in the total cholesterol, which adds to the validity of the observations in the experimental group. However, the control group has shown a significant rise in VLDL cholesterol, which is difficult to explain, specially in the absence of a rise in triglyceride levels. There is also a trend towards a decline in LDL cholesterol in the control group.

An interesting observation in both the experimental and control groups is that, except in case of the changes in LDL levels in the control group, the changes are more marked at 4 wk than at 8 wk (Fig. 2). This points to homeostatic regulation which tends to bring the lipoprotein profile back to the

baseline which the individual has presumably been having for a long time as a product of his genetic constitution, diet and environmental factors. However, in case of the control group, LDL levels are lower at 8 wk than at 4 wk (Fig. 2). Although the levels at both 4 wk and 8 wk are not significantly different from those at 0 wk, the trend suggests that a diet in which visible fat is strictly restricted to mustard oil may have a sustained LDL-lowering effect. This is possibly due to the high PUFA and MUFA content, and negligible SFA content of mustard oil (14).

Analysis of the subgroups showing greater than 15% rise in total cholesterol at 4 wk essentially confirms the above conclusions. In this subgroup also, the experimental subjects have the rise in serum cholesterol mainly due to a rise in HDL cholesterol, and the rise is more marked at 4 wk than at 8 wk. On the other hand, the 5 control subjects in this subgroup have shown an anamolous transient rise in total cholesterol at 4 wk which is primarily due to a rise in LDL cholesterol. This unfavourable change while on a diet in which visible fat was restricted to mustard oil is difficult to explain on the basis of the diet, and may be due to mental stress (20). If this subgroup is excluded, the remaining 28 subjects of the control group show, in contrast, a significant fall in LDL (88.2±24.8 vs. 95.5±21.8 mg/dL) and a significant rise in HDL (43.9±6.7 vs. 39.8±4.4 mg/dL) at 4 wk as compared to 0 wk. However, these changes do not persist at 8 wk, pointing to the genetic factors being the dominant determinant of the lipoprotein response (21).

The absence of any effect on Lp(a) levels is consistent with the known resistance of this lipoprotein fraction to dietary changes (22). The trend towards a fall in MDA levels in both the experimental and control groups suggests a decline in lipid peroxidation. Although it may indicate the favourable result of a better regulated diet during the study, it would be presumptuous to read too much in this change.

In spite of randomization, the HDL cholesterol levels in the experimental group were higher than in the control group at –2 wk (Tables II and III). However, the two groups became comparable in this respect by 0 wk. Thus the lead-in period of 2 wk seems to be necessary and sufficient, as was observed also by Ghafoorunissa (16).

In short, the major conclusions of the present study are that ghee has a tendency to raise serum cholesterol, but this effect is primarily due to a rise in HDL cholesterol. Hence ghee does not have any significant effect on the total cholesterol/HDL cholesterol ratio. On the other hand, if visible fat is restricted to mustard oil, it has a tendency to lower LDL cholesterol. A judicious combination of mustard oil and ghee is thus an acceptable option, as in the experimental group of the present study.

Only limited conclusions can be drawn from the present study because it has been done on healthy, young, physically active volunteers following a healthy lifestyle in an ashram. More studies arc required on older subjects, hyperlipidaemic subjects, and in those following less healthy lifestyles. Further studies are also required to investigate the effects of ghee on other related variables such as platelet aggregation and oxidative modification of LDL. In view of the findings in the control

group, it would also be worthwhile to study mustard oil for a period longer than 8 wk for its effects on LDL levels.

ACKNOWLEDGEMENTS

We would like to thank the Indian Council of Medical Research for financial support (ID No. 2000-00810). Ms. Promila Kapoor, Mr. Rakesh Kumar, Ms. Suman Thakur and Mr. Pawan Kumar provided able technical assistance, and Mr. Satish Sachdeva efficient secretarial assistance. We are grateful to all the colleagues who helped with collection of blood samples, to all the volunteers for their cooperation, and to Sri Aurobindo Ashram (Delhi Branch) for allowing us to do the study and extending all facilities.

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