EFFECT OF SUBSTITUTION OF HYDROGENATED OIL BY A MIXTURE OF GROUNDNUT OIL AND HYDROGENATED OIL

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The use of a mixture of 60% groundnut oil and 40% hydrogenated oil in the peace time Army ration, has been found to maintain the serum lipids at a lower level and improve the physical efficiency of albino rats.

Hydrogenated oil constitutes the major source of dietary fat for the Armed Forces. Although it is considered suitable from the point of view of logistics, nutrition and culinary practices, it is very low in polyunsaturated fatty acids (PUFA). Significant correlations have been established between PUFA content of the dietary fat, hyper cholesterolaemia and atherosclerosis[1,2,3,4]. A high cholesterol level in blood has been associated with coronary diseases (CHD) and it has been considered one of the major risk factors for the development of CHD.

The PUFA content of hydrogenated oil issued to the Armed Forces is of the order of 2% and its biological availability is limited to a great extent by losses due to auto-oxidation during storage and polymerisation in the processes like deep fat frying etc. The optimum level of fat or the PUFA in human diet has not yet been precisely defined . However, the American Heart Association has recommended that saturated fats should not contribute more than 10% of the total calories in a dietary regimen where the caloric contribution of fat is not more than 30% of the total calories[5]. Judged by these standards again, the level of PUFA in the Army ration appears to be definitely on the lower side.

In a large scale users' acceptability trial carried out by this laboratory[6] earlier, it was found that refined groundnut oil fortified with Vitamin A was acceptable to Armed Force personnel, deployed under different climatic conditions, up to a maximum of 60% of the total entitlement. The replacement of 60% of hydrogenated oil by refined groundnut oil will provide 12–13% PUFA on total fat basis which has also been found sufficient for minimising hypercholesterolaemia[4]. The present communication deals with the effects of the above replacement of on the serum and the liver lipid patterns and physical efficiency of albino rats.

EXPERIMENTAL PROCEDURE

Experiments were carried out in three series.

In series I, twenty four laboratory bred male albino rats, weighing 220 to 240 grams each, were divided into two equal groups 'A' and 'B' of twelve animals each and housed in separate cages. For collecting the base line data for the serum lipids 1.0 to 1.5 ml of blood was drawn under slight anaesthesia by heart puncture in the fasting state. The serum was separated and the analysis was completed on the day of collection. The animals of group 'A' were kept on the normal Army ration and served as control for those of group 'B' fed on the modified Army ration. The animals were weighed each week and the weekly intake of food was determined on dry basis (Table 1).

Blood was collected in the fasting state at the end of the fourth, eighth, and twelfth week for the estimation of the serum lipids. At the end of the eleventh week, a swimming test was carried out to determine

Table 1

Weekly Average food consumption on dry basis over a period of twelve weeks

Week/ Group	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	Mean ± SD 4 weeks	8 weeks	12 weeks
A	80	84	93	94	88	97	90	97	88	91	93	71	87·8±5·9	90·4±5·7	88·9±7·2
B	83	100	84	88	86	75	97	86	81	92	95	93	88·8±6·8	89·9±6·0	90·0±6·1

The difference between the means of the two groups in 4, 8 and 12 weeks is not significant. $P < 0.05$.

the physical efficiency. On completion of the trial period of twelve weeks, the animals were sacrificed and the samples of liver were collected and analysed.

In series II, two groups of male albino rats weighing 220 grams each, maintained on stock diet were fed *ad libitum* on normal Army ration and modified Army ration, supplemented with cholesterol at 1.5 % level for a period of twelve weeks. Serum cholesterol levels were determined both at the commencement and at the end of the experiment.

In series III, sixteen male weanling rats were divided into two groups of eight each. These groups were fed on normal Army ration and modified Army ration for 52 weeks. Physical efficiency in these groups was assessed at the end of 42nd and 52nd week.

Preparation of the ration: Wheat flour, Bengal gram flour, salt, milk powder and about 80% of the fat were mixed together as uniformly as possible. The vegetables cooked by boiling in water were added to the above mixture to prepare a dough. Chapaties were prepared by baking. The onions and boiled vegetables were chopped and fried in the remaining 20% of the fat; sugar, cut sweet lime and chapaties torn into small pieces, were mixed and the mixture was passed through a mincer. The material, sufficient to last for one week, was packed in paper foil laminate pouches and stored in the deep freeze. The proximate analysis is shown in Table 2.

TABLE 2

COMPOSITION OF THE EXPERIMENTAL RATIONS

	Normal Army ration (for rats of group 'A') (gm)	Modified Army ration (for rats of group 'B') (gm)		Normal Army ration (for rats of group 'A') (gm)	Modified Army ration (for rats of group 'B') (gm)
Wheat flour	600	600	Potato (fresh)	110	110
Bengal gram flour	90	90	Onion	60	60
Whole milk powder	44	44	Fruits (Sweet lime)	50	50
Sucrose	90	90	Oil hydrogenated	70	—
Salt	20	20	60% groundnut oil and 40% hydrogenated oil mixture	—	70
Vegetable fresh (Carrots)	180	180			

Proximate analysis (%): Moisture—40·5, dried feed—59·5, Fat—9·5, Protein—20·2, Carbohydrate—63·4, Ash—1·8 and Crude fibre—5·1.

Assessment of physical efficiency: 0.5 ml of blood was drawn in the fasting state from each animal under slight anaesthesia by heart puncture and the fasting lactic acid level was determined according to Barker and Summerson[7]. The rats were kept at rest for one hour and then kept in the swimming tank for one hour. 0.5 ml of blood was again collected at the end of the test and the lactic acid level re-estimated.

Determination of serum lipids: The blood, after collection was allowed to clot at room temperature and the serum separated by centrifuging. The analysis was completed on the day of collection. The total cholesterol was estimated according to Searcy & Bergquist[8]; Phospholipids according to King & Wootton[9]; and Total esterified fatty acids (TEFA) and triglycerides according to Reinhold, Yonan & Greshman[10].

Determination of liver lipids: The specimen of liver was weighed and dried at 80° to 90° C to constant weight. The dried material was then powdered and kept in the deep freeze. The following method was adopted for estimation of liver lipids. The analysis was completed in a week.

0.5 to 0.8 grams of the dried liver was extracted with petroleum ether (40°–60°C) in a Soxhlet apparatus. After removing the solvent, the residual lipid was weighed, dissolved in chloroform and solution made upto 100 ml with the same solvent. For the estimation of total cholesterol, phospholipids and TEFA 0.5 ml of chloroform solution were separately evaporated to dryness and the methods for the serum were followed. Free cholesterol was determined by the method of Searey & Bergquist[8] after evaporating 1 ml of the chloroform solution to dryness. The value of triglyceride was calculated according to the recommendation of the American Association of Clinical Chemists. The figures for esterified cholesterol were obtained by subtracting the values of free cholesterol from those of the total cholesterol. The data of various parameters were statistically analysed. The significance of the difference of the means was calculated according to the students' 't' test (P less then 0.05).

RESULTS AND DISCUSSION

It is observed from Table 3 that the pattern of changes in the various serum lipids in the normal Army

TABLE 3

SERUM LIPIDS IN THE TWO GROUPS OF RATS KEPT ON THE RATIONS

Group	Week	No. of rats	TEFA (meq/lit) Mean±SD	Cholesterol (mg/100 ml) Mean±SD	Phospholipid (mg/100 ml) Mean±SD	Triglyceride (mg/100 ml) Mean±D	C/P ratio Mean±SD
A	initial	12	5·8±1·9	61·2±10·3	135·9±37·6	82·6±57·0	0·48±0·13
	4th	12	6·8±1·9‡	53·0±11·2	151·6±38·4	108·4±37·5	0·38±0·11
	8th	9	14·6±2·1†‡	68·9± 8·4	118·2±12·1	298·1±39·17†	0·61±0·10†
	12th	7	8·3±0·83†	60·8±11·9	94·3± 16·2†	160·2±25·0†	0·65±0·13†
B	initial	12	5·8±1·9	61·2±10·3	135·9± 37·6	82·6±57·0	0·48±0·13
	4th	12	4·8±0·9*‡	62·3±12·5	159·5± 19·4	34·2±18·2‡	0·43±0·12
	8th	11	7·5±1·6†‡	64·3±12·9	107·7± 15·6†	129·9±42·1†‡	0·60±0·13†
	12th	8	8·8±1·9†	58·1± 5·1	86·6± 6·4†	181·7±51·8†	0·68±0·09†

*Only four samples of serum were analysed.
† Significantly different from the preceding value.
‡ Significantly different from the values of group 'A'.

TABLE 4

LIVER LIPIDS OF RATS FED ON ARMY RATION AND MODIFIED ARMY RATION FOR 12 WEEKS

No. of rats fed on modified ration =8 No. of rats fed on Army ration =7

	Army ration	Modified Army ration
Moisture (gm/100 gm)	69·80±1·20	68·80±1·40†
Total lipids (gm/100 gm)	5·77±2·10	7·49±2·12†
TEFA (meq/100 gm)	10·03±1·74	17·15±3·40*
Total cholesterol (mg/gm)	3·66±0·46	4·81±0·87*
Free cholesterol (mg/gm)	2·22±0·38	2·23±0·54†
Phospholipids (gm/100 gm)	1·76±0·16	2·74±0·70*
Triglycerides (gm/100 gm)	1·82±0·34	2·70±1·55*

†Not significant. *Significant.

TABLE 5

SERUM CHOLESTEROL AFTER A FEEDING PERIOD OF 12 WEEKS

Group	Week	No. of rats	Cholesterol (mg/100 ml) Mean±SD	Significance of the difference of the mean < P-0·05
Army ration with 1·5% cholesterol	initial	6	59·5±3·2	Highly Significant
	12th	6	132·8±13·7	
Modified Army ration with 1·5% cholesterol	initial	6	59·5 ±3·2	
	12th	5	103·7±11·6	

ration and the modified Army ration are almost similar. The values for TEFA and triglycerides in the case of Army ration remained at a higher level throughout the study upto 8th week and the concentration of TEFA, triglycerides and C/P ratio in both the groups showed a significant elevation at the end of feeding for 8 weeks. At the end of 12 weeks TEFA and triglycerides in group B showed a decline while those for group A showed a significant rise; however the means of these values for group A and B were not significantly different.

No significant changes were found in cholesterol concentrations between the two groups throughout the study, although slight variation was found within the groups. The concentration of phospholipid in each group tended to rise at four weeks, and thereafter declined gradually to below the initial levels, at the end of 12 weeks, the differences between the groups were not significant at any stage. Thus the comparison of the figures for the various serum lipids indicated that the rats of groups A and B varied in a similar manner, the notable difference being that TEFA and triglyceride levels of group B tended to remain significantly at lower levels as compared to the other group.

No significant difference is observed in the intake of food by the two groups (Table 2) and thus the difference in the lipid levels between the two groups is due to the difference in the nature of the fat consumed.

The data recorded in Table 4 indicate that after a period of 12 weeks, the livers of the rats of group 'A' kept on the normal Army ration show significantly lower levels of total cholesterol, TEFA, triglycerides and phospholipids than the rats of group B fed on modified Army ration.

TABLE 6

BLOOD LACTIC ACID LEVEL (*mgm*/100 *ml*) BEFORE AND AFTER SWIMMING EXERCISE IN SERIES I, II AND III

Series	Group	No. of rats	Before exercise Mean±SD	After exercise Mean±SD
I	A	7	13·60±4·30	37·70±8·30
	B	9	13·80±3·20	23·80±4·80*
II	A	8	18·58±2·97	40·48±5·50
	B	8	20·31±1·13	34·55±2·02*
III	A	5	18·80±1·33	31·29±2·72
	B	8	17·86±1·08	25·36±0·67*

*Significant ($P < 0.05$).

There is no significant difference in serum cholesterol levels between the rats of group A and B throughout the trial (Table 3). It is observed from Table 5 that on supplementation of the diet with 1.5% cholesterol, a highly significant difference exists in the serum cholesterol level of the two groups of rats after a period of twelve weeks, the ration containing the hydrogenated oil as the source of fat being decidedly more hypercholesterolaemic than that containing a mixture of hydrogenated oil and groundnut oil as the source of fat.

The results of the test of physical efficiency (Table 6) indicate a significantly lower level of blood lactic acid after exercise in the rats of group 'B' than in the rats of group 'A' . This trend of improved physical efficiency was observed in rats maintained on modified Army ration during 11 weeks, 42 weeks and 52 weeks. The increased concentration of PUFA in the modified Army ration seems to improve the physical efficiency of the rats. A similar observation was recorded by Scheer[11] and co-workers with rats fed on varying quantities of cotton seed oil containing 1% linoleic acid. They had observed an increase in the swimming time of the rats with an increase of the linoleic acid containing cotton seed oil in the diet.

CONCLUSION

The use of a mixture of 60% groundnut oil and 40% hydrogenated oil in place of only hydrogenadted oil in Army ration has been found to maintain serum lipids at a lower level.

The rats fed on the modified Army ration show a higher physical efficiency, as observed by the swimming test, than those fed on the normal Army ration.

The livers of rats maintained on the modified Army ration, for a period of 12 weeks, contain a higher concentration of cholesterol, TEFA, triglycerides and phospholipids than those of rats fed on the normal Army ration for the same period.

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