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Protein deficiency and age related alterations in rat peritoneal macrophage lipids

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Abstract. The effects of dietary protein restriction and age on the thioglycollate elicited peritoneal macrophage lipid constituents were studied. Impact of subtle changes in lipid components on macrophage functions have been assessed. Lipid profiles of macrophages recovered from rats fed 20 and 4% protein diets and stock diet fed rats (0 and 3 wk) were comparable qualitatively. Quantitative analysis however revealed significant decrease in phospholipids (30–40%) and consequent elevation of cholesterol/phospholipid molar ratios in the protein depleted and young rats (0 wk), compared to the protein fed groups. The protein deficient and the young rats also exhibited accumulation of certain neutral lipids and reduction in triglycerides. Analysis of fatty acid methyl esters of macrophage phospholipids revealed the predominance of long chain polyunsaturated fatty acids even when oleic ($C_{18:1}$) and linoleic ($C_{18:2}$) formed the bulk of unsaturated fatty acids in the diet. However, the long chain poly unsaturated fatty acid content, particularly the docosahexaenoic acid ($C_{22:6n-3}$) was greatly reduced in the protein depleted and 0 wk rats. Observed changes in the long chain polyunsaturated fatty acids of macrophage phospholipids may be of physiological significance as they modulate the immunological functions of the cell.

Keywords. Protein deficiency; peritoneal macrophages; lipids; cholesterol/phospholipid ratio; fatty acid composition.

Introduction

Macrophages rank among the most important cells which guarantee the optimum course of immune reactions. Recently much attention has been paid to the role of dietary fat in immune response (Smith *et al.,* 1985). Macrophage mediated immune functions such as phagocytosis and cytotoxicity are regulated by critical composition of the cell membrane glycolipids (Schlager *et al.,* 1983). Protein restrictt-ion has adverse effects on various aspects of immune competence (Salimonu *et al.,* 1982). Though proteins and lipids are essential components of cell membranes, many of the membrane functions have been traditionally associated with proteins. However, there is sufficient evidence to suggest that lipids also greatly influence the cellular functions (Spector and Yorek, 1985). In macrophages the membrane associated endocytic functions apart from being receptor mediated, are known to be governed by the fluidity of the membranes (Mahoney *et al.,* 1980). The physical state of the membrane lipids can modulate the physiological function of the membranes. Various reports have suggested a role for lipids and their

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Abbreviations used: PBS, Phosphate buffered saline; FAME, fatty acid methyl esters; TLC, thin-layer chromatography; GLC, gas-liquid chromatography; PUFA, polyunsaturated fatty acids; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid.

components, particularly fatty acids, in the process of macrophage activation (Mahoney *et al.,* 1977; Schroit and Gallily 1979). Age and dietary manipulations are known to cause variations in the cellular components which govern their immune functions (De La Fuente, 1985; Smith *et al.,* 1985). However, these effects are not well elucidated. The present studies were carried out to characterise the changes in the macrophage lipid profile, particularly in their fatty acyl chains in response to protein deficiency and underdevelopment.

Materials and methods

Animals and diet

Male weanling Wistar rats weighing 40–50 g were fed stock diet (table 1) *ad libitum* and on reaching about 100 g body weight, were separated randomly into 4 groups of 10. One group of animals was injected intraperitoneally with 5 ml of 3% thioglycollate (Difco product). Three days later, they were sacrificed by anesthetizing with ether and macrophages were isolated. Remaining 3 groups of animals were each fed *ad libitum* isocaloric stock, 20 and 4% protein diets (table 1) respectively. The low protein diet was nutritionally adequate except for protein levels. Each animal was housed separately in a hanging type cage and its food intake and body weight were recorded. Animals had continuous access to food and water in a temperature and light controlled room (23–25°C, 12 h light-dark cycle). At the end of 3rd week the animals were sacrificed on the 3rd day following thioglycollate injection.

Table 1. Composition of diets.

Stock diet		Experimental diet (% protein)		
Ingredient	Amount (%)	Ingredient	20 amount %	4 amount %
Cracked wheat	70	Casein	20	4
Cracked Bengal gram	20	VSD	2	2
Fish meal	5	Salt mixture	4	4
Yeast powder	4	Sugar	9	9
Shark liver oil	1	Seasame oil (til oil)	6	6
		Corn starch	59	75

The experimental diets were designed to be isocaloric to stock diet which provided about 350–360 cal/100 g of diet.
100 g of VSD (vitaminised starch dextrose) comprised of all water soluble vitamins totalling up to 12·14 g, 37·86 g sugar and 50 g starch. Seasame (til) oil (6 g) was fortified to contain 2000 IU, vitamin A palmitate, 1000 IU ergocalciferol and 5 mg dl-α-tocopherol acetate.

Isolation of macrophage

Peritoneal macrophages were isolated (Edelson *et al.,* 1975) using cold 0.1 M phosphate buffered saline (PBS), pH 7.2. The collected peritoneal fluid was

centrifuged at 600 *g* for 10 min at 4°C. Peritoneal cells were freed of contaminating erythrocytes with hypotonic NH_4C1 (0·85%). The resultant pellet was transferred into sterile plastic petridishes (9·5 cm diameter) and suspended in Eagles minimum essential medium (Difco product), containing 100 μg/ml each of penicillin and streptomycin and incubated for 2 h at 37°C. The nonadherent cells were removed by washing 4–5 times with saline. The adhering cells were scrapped with a rubber policeman and suspended in saline. A giemsa stained preparation of this suspension was observed under light microscope and 95% of these cells were identified as macrophages, almost free of lymphocytes and other cells. Cells were counted at 10X using a hemocytometer.

Isolation of macrophage membrane

Macrophage membrane was isolated by using Tris-HCI buffer pH 7·7 essentially as described by Snyderman *et al.* (1984). To assess the membrane purity, 5′-nucleotidase activity was estimated using adenosine monophosphate as the substrate (Widnell and Unkeless, 1968).

Lipid analysis

The cells were made 0·1% with Triton X-100 and homogenised and proteins were estimated according to the method of Miller (1959). The cells from 5 rats were pooled and the lipids were extracted with chloroform: methanol 2:1 (v/v) as described by Folch *et al.* (1957). The extracts were pooled, evaporated under N_2 and total lipids estimated colorimetrically (Bragden, 1951). Lipids were separated on thin-layer chromatography (TLC) using hexane: diethylether: acetic acid [85:15:1 (v/v)]. Lipid containing regions were visualized by exposure to I_2 vapours and the separated fractions were scrapped and eluted with chloroform and estimated by specific colorimetric methods. Membrane lipids were also similarly extracted and analysed. Cholesterol and cholesterol esters were estimated by the procedure outlined by Sperry and Webb (1950). Triglycerides were estimated using chromotrophic acid reagent (Lambert and Neish, 1950). Fatty acids were estimated as described by Duncombe (1963). Phospholipids were estimated (Charles and Stewart, 1980) using lysophosphatidyl ethanolamine as the standard.

Fatty acid analysis and gas chromatography

Total phospholipids were eluted and fatty acid methyl esters (FAME) were prepared by transesterification (Mahoney *et al.,* 1977). Phospholipid samples (100 μg) were heated with 300–400 μl of 6% HCl in methanol and heated for 16 h at 80°C under N_2. After evaporating excess methanolic HCl, the residue was dissolved in a small volume of water (0·5 ml) and methyl esters were extracted with equal volume of distilled hexane thrice. The pooled extracts were evaporated and redissolved in hexane and analysed. The stock diet fat (obtained by Soxhlet extraction) and seasame oil (400 μg each) used in the prepared diets were directly transesterified and their total FAME were analysed.

Gas-liquid chromatography (GLC) analysis was done by temperature programming in a Shimadzu GC-7A model gas Chromatograph. The column (6 ft. × 3 mm) was

packed with 10% Silar 7C. The initial temperature was 140°C and it was gradually increased at 4°C/min, after initial 4 min hold up to the final temperature of 210°C till the end of the analysis (40 min). The temperature of the injection port was 230°C. The results are reported as per cent composition of each FAME compared with the total identified fatty acid methyl esters. The following FAME standards obtained from Sigma Chemical Co., St. Louis, Missouri, USA, in order of their elution were examined. 9:0, 12:0, 14:0, 16:0, 16: $1_{n-7,}$ 18:0, 18:$1_{n-9,}$ 18: $2_{n-6,}$ 20:0, 18:3_{n-3}, 20:1_{n-9}, 20:3_{n-9}, 22:0, 20:4_{n-6}, 22: 1_{n-9}, 20:5_{n-3}, 24:0, 24:1, 22:5_{n-3} and 22:6_{n-3}. The position of the double bond is given when known with the omega classification.

Results and discussion

Growth profile of animals

The rats fed 20% diet gained weight (both total body wt. and wt./g of diet), in a linear pattern during the experimental period. The immediate physiological response due to protein restriction reflected in a reduction in weight which remained constant, till the end of the experiment. Their body weights were comparable to those of the young rats used in the 0 wk control group. The food intake and weight gain pattern of the stock diet fed, 3 wk control rats was similar to those of the 20% protein fed rats.

Macrophage cell count

Macrophages recoverable from the 20% protein and stock diet fed rats, possessing a normal linear growth pattern in response to thioglycollate stimulation was higher. The total cell count per rat was reduced by about 62% in the 4% protein fed rats compared to the 20% protein fed animals (figure 1). The young rats (0 wk control) had 41 % lower cells than the 3 wk control rats. Protein per 10^6 cells was comparable in all the 4 groups. However the total lipid per 10^6 cells was nearly 2-fold in the protein deficient and young animals compared to the other 2 groups. Consequently lipid to protein ratio was about 1 in the 20% protein and 3 wk stock diet fed rats. It increased to 2·4 in the 4% protein and 0 wk stock diet fed groups.

Macrophage lipid profile

Starting from $1{\cdot}222 \times 10^8$ cells, a membrane preparation exhibiting 3·2-fold purification compared to the crude homogenate, as indicated by 5′-nucleotidase activity was obtained. The recovery in terms of protein was only 22·7% though in terms of enzyme activity it was 72·8%. However the yield of membrane fraction in terms of protein was only 7·9 $\mu g/10^6$ cells. In the initial standardisation experiments it was observed that the TLC profiles of lipid fraction of the whole macrophages and isolated membrane were identical. The percentage distribution (table 2) of individual lipids were comparable. Slight decrease in cholesterol and cholesterol ester and increase in phospholipids in the membrane compared to whole cells,

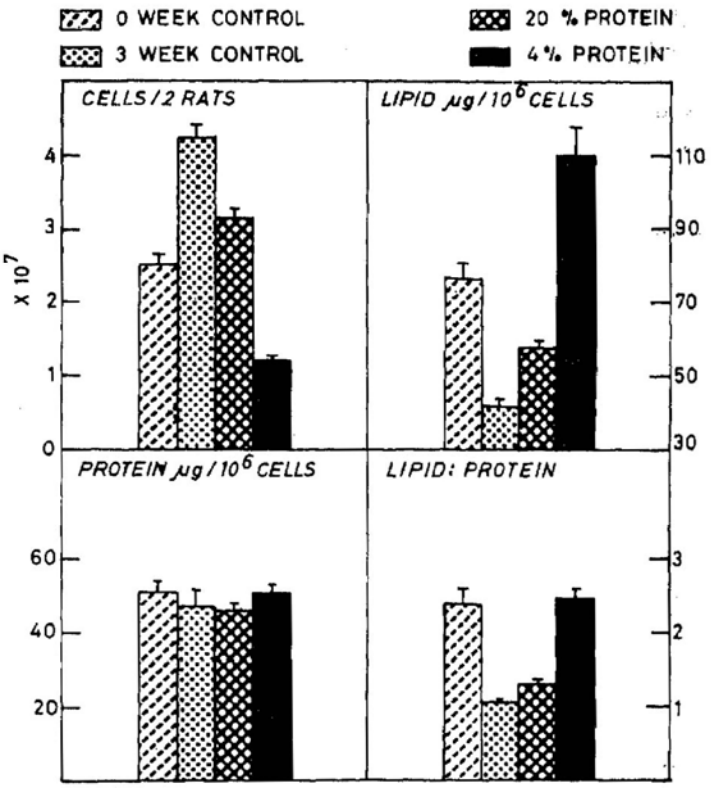

Figure 1. Cell count and composition of thioglycollate elicited macrophages. The cells were isolated from the peritoneal cavity by cold PBS washing and incubated in sterile petridishes. The non adherent cells were washed away and the adherent macrophages recovered and counted. The cellular protein and lipid contents were estimated.

Table 2. Comparison between lipid composition of macrophage whole cells and membrane.

	Distribution of lipids (%)	
Lipid component	Macrophage membrane	Macrophage cells
Cholesterol	8·28	10·76
Cholesterol ester	2·17	3·16
Phospholipid	48·5	43·25
Triglycerides	6·2	7·9
Free fatty acids	10·28	11·75
Other lipids	24·56	23·18
Lipid/protein ratio	1·92	0·6
Cholesterol/phospholipid	0·45	0·67

Values are mean of duplicate experiments. Ten rats were used in each set. The lipids were extracted, separated and quantitated as detailed in the text.

reflected in decreased cholesterol/phospholipid molar ratio of the membrane. The membrane preparation required large number of cells. However, the differences

were very little in absolute terms. Hence in the subsequent studies dealing with experimental animals, where the results were comparative, whole macrophages were directly used for lipid extraction.

The profile of lipids (figure 2) was identical in all the groups, though they varied

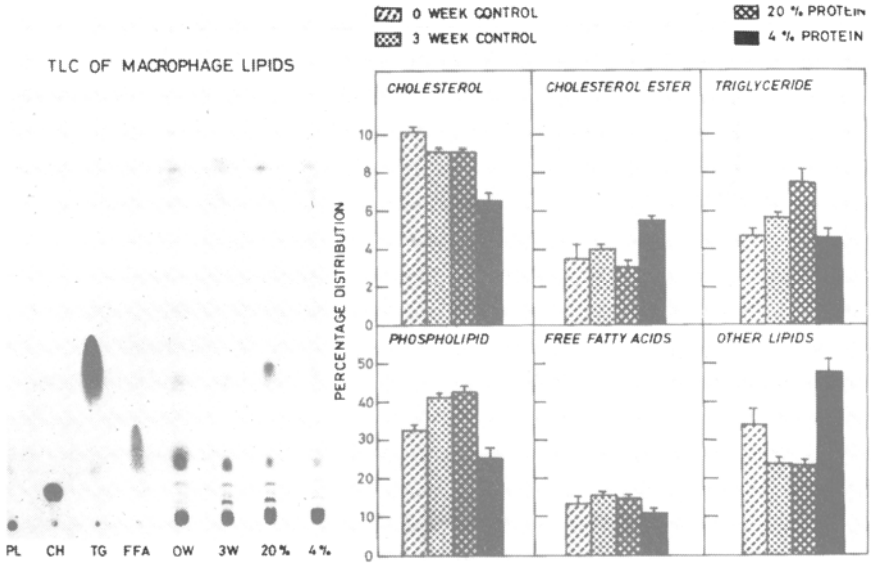

Figure 2. Distribution of macrophage lipid components. Lipids separated by TLC were visualized by exposure to iodine vapours. The lipid spots were scrapped and then eluted with chloroform. The components were estimated by specific colorimetric methods. Values represent mean ± SD of 8 independent estimations and are expressed as per cent of total.

quantitatively. Phospholipids were reduced by 30–40% in the protein restricted and 0 wk groups compared to the other two groups. Cholesterol was slightly lower in the 4% group which was compensated by the corresponding increase in the cholesterol ester fraction (table 3). Consequently the cholesterol/phospholipid molar ratio was 0·67 and 0·63 in the 3 wk and 20% groups while it was about 1·0 in the other two groups. This increase would alter membrane fluidity and permeability factors that govern functional properties of macrophages.

Table 3. Macrophage cholesterol and phospholipid content.

Groups	Cholesterol	Cholesterol ester	Total cholesterol	Phospholipid	Cholesterol: phospholipid molar ratio	
	(μg/mg protein)					
0 wk control	23·9 ± 2·4	8·44 ± 2·26	32·35 ± 4·53	76·13 ± 13·46	0·91 ± 0·04	$P \leq 0{\cdot}0001$
3 wk control	30·09 ± 3·62	12·94 ± 1·34	43·03 ± 4·74	134·49 ± 16·66	0·67 ± 0·01	
20% protein	30·09 ± 2·99	11·25 ± 2·0	41·34 ± 4·96	137·57 ± 18·16	0·63 ± 0·03	$P \leq 0{\cdot}0001$
4% protein	16·87 ± 1·10	17·15 ± 6·07	34·02 ± 7·03	67·23 ± 15·15	1·07 ± 0·03	

The result are expressed as mean ± SD of 8 independent estimations. The contents are expressed as μg/mg of total macrophage protein

Triglycerides were more in the protein fed groups but free fatty acids did not differ significantly. The other lipid fractions comprising of the mono and diglycerides and unidentified lipids increased by about 40–100% in the young and protein restricted groups. Schmidt *et al.* (1984) have shown active turnover of phospholipids in macrophages during their activation. The synthesis of these lipid components are enzymatically mediated. Significant protein deficiency induced changes in rat liver membrane lipids have been reported earlier (Gerson, 1974).

Macrophage phospholipidfatty acids

The FAME analysis of the dietary fat (figure 3) indicated that $C_{18:1}$ and

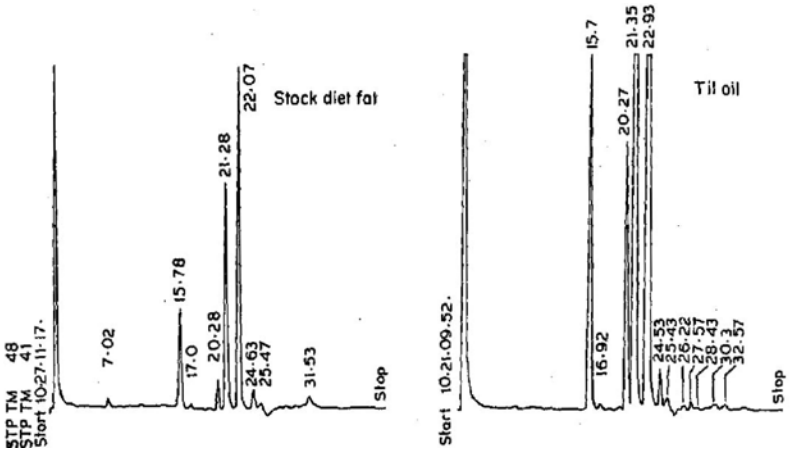

Figure 3. Separation of dietary fatty acids. Dietary fats were directly transesterified with methanolic HCl and total fatty acid methyl esters were analysed by GLC as described under methods. The major fatty acid peaks were identified as C_{16}(15), C_{18}(20), $C_{18:1}$ (21), $C_{18:2}$(22), $C_{18:3}$(24) and $C_{20:1}$(26). The numbers in parentheses represent their retention time in min.

$C_{18:2}$ constituted about 80% of the total fatty acids (table 4). The saturated fatty acids C_{16} and C_{18} were about 15%. Remaining fatty acids represented less than 5%. The fatty acid profile of the macrophage phospholipids was qualitatively comparable to those obtained by temperature programmed GLC analysis reported for mice resident peritoneal macrophages (Leslie *et al.,* 1985). The macrophage phospholipids in all the groups (figure 4) comprised of low levels of $C_{18:1}$ and $C_{18:2}$ (less than 12%, table 5), though they were abundantly present in the diet (table 4). This is quite intriguing as macrophage fatty acids are known to be modulated by dietary fats (Leslie *et al.,* 1985). The total saturated fatty acids (table 6) were 14–18% in the 3 wk control and 20% protein fed rats. It was higher (26%) in the 0 wk control and protein restricted groups. Macrophage phospholipids isolated from the 3 wk control and 20% protein fed rats contained very high amounts of long chain polyunsaturated fatty acids (PUFA) compared to the values reported for resident peritoneal macrophages (Leslie *et al.,* 1985). Possibly the increased long chain PUFA may be reflecting an activated state of macrophages. The long chain PUFA, particularly the docosahexaenoic acid (DHA, $C_{22:6n-3}$) was

Table 4. Fatty acid composition of dietary lipids.

Fatty acids	Til oil	Stock diet fat
	% wt. of total fatty acids	
C_{12}	ND	⩽0·01
C_{16}	8·86	13·48
$C_{16:1}$	0·12	⩽0·01
$C_{18:0}$	5·79	3·85
$C_{18:1}$	38·42	30·66
$C_{18:2n-6}$	43·35	47·23
$C_{18:3n-3}$	1·52	2·35
$C_{20:1n-9}$	0·71	1·11
$C_{20:3n-9}$	0·14	ND
$C_{20:4n-6}$	0·36	ND
$C_{22:1n-9}$	0·19	⩽0·01
$C_{20:5n-3}$	0·05	1·287
C_{24}	0·30	ND
$C_{24:1}$	0·16	ND

Stock diet fat was obtained by Soxhlet extraction for 18 h using petroleum ether (B.Pt. 60–80°C). The fats were directly transesterified and FAME analysis done by GLC as described in the methods.
ND, Not detected.

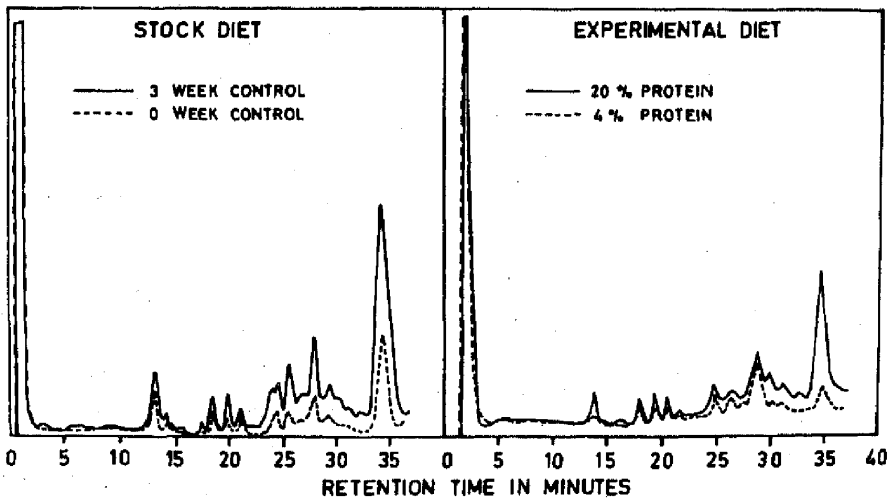

Figure 4. Fatty acid profile of macrophage phospholipids. Macrophage total phospholipids (100-150 μg/sample) eluted from TLC plate were transesterified and FAME analysis done by GLC. The profiles obtained with the 4 different groups were compared. The retention time for the major fatty acids identified in the samples in min (given in the parentheses) were as follows: C_{16}(14–15), C_{18}(19–20), $C_{18:1}$(21), $C_{18:2}$(22), $C_{18:3}$(24), $C_{20:1}$(26), $C_{20:3}$(27), $C_{20:4}$(28), $C_{20:5}$(30), C_{24}(31), $C_{22:5}$(32) and $C_{22:6}$(34–35).

reduced in the 0 wk control and protein restricted groups, compared to the other two groups (table 5). These values are more similar to that reported for the non elicited macrophage phospholipid fatty acids (Leslie *et al.*, 1985). The macrophages isolated from the protein deficient group contained significantly higher arachidonic

Table 5. Fatty acids of macrophage phospholipids.

	Control (wk)		Protein (%)	
Fatty acids	0	3	20	4
	% wt. of total fatty acids			
C_{16}	17·08 ± 3·84	10·31 ± 0·62	13·98 ± 1·76	16·61 ± 7·17
C_{18}	6·72 ± 1·4	3·78 ± 0·68	4·99 ± 1·88	6·73 ± 1·26
$C_{18:1}$	5·42 ± 1·9	4·04 ± 1·09	5·18 ± 0·68	6·66 ± 2·26
$C_{18:2n-6}$	7·07 ± 1·89	5·56 ± 1·69	4·22 ± 1·35	7·0 ± 0·22
$C_{18:3n-3}$	3·92 ± 1·74	1·22 ± 0·49	1·21 ± 0·56	2·88 ± 0·58
$C_{20:1n-9}$	2·07 ± 1·13	1·29 ± 0·56	1·66 ± 1·6	3·42 ± 1·06
$C_{20:3n-9}$	4·35 ± 0·9	6·96 ± 2·75	4·05 ± 0·97	6·9 ± 2·3
$C_{20:4n-6}$	12·27 ± 4·17	10·51 ± 2·15	14·31 ± 2·85	18·19 ± 2·0
$C_{22:1n-9}$	2·78 ± 0·87	1·38 ± 0·56	1·19 ± 0·17	2·85 ± 0·97
$C_{20:5n-3}$	14·30 ± 4·76	12·27 ± 1·42	11·62 ± 3·97	11·79 ± 3·29
C_{24}	3·00 ± 1·08	ND	ND	3·22 ± 0·39
$C_{22:5n-3}$	5·65 ± 3·78	1·15 ± 0·68	ND	3·12 ± 1·57
$C_{22:6n-3}$	15·34 ± 4·46	42·42 ± 3·52	37·53 ± 6·11	10·54 ± 1·39

Values are mean ± SD of 4 sets of samples per group, analysed in duplicates. Each experimental sample consisted of lipids extracted from 5 rats. Data are reported as per cent composition of the total identifiable fatty acids.

acid ($C_{20:4n-6;}$ $P \leq 0{\cdot}013$) than the other 3 groups. The unsaturation index and unsaturation index/saturated ratio were much lower in the 0 wk and protein restricted groups (table 6). The n6/n3 ratio was higher in these two groups.

The long chain PUFA are ubiquitous and essential constituents of structural lipids. They are derived from their precursors $C_{18:1}$, $C_{18:2}$ and $C_{18:3}$ by sequential desaturation and chain elongation, mediated by Δ^6, Δ^5 and Δ^4 desaturates (Holman, 1986). Protein and essential fatty acid deficiency induced changes in their activities in rat livers have been observed (De Tomas *et al.,* 1980). Recently Narce *et al.* (1988), have shown that in growing rats, on a low protein diet, the fall in hepatic Δ^6 and Δ^5 desaturase activities lead to reduced formation of C_{20} and C_{22} fatty acids. However the changes were reversed on balanced refeeding, implicating them to be direct effects of protein deficiency, Results presented here indicate the presence of active desaturases in macrophages, warranting separate studies on the biosynthesis of PUFA from their labelled precursors in protein restriction to conclusively assess the impact on these enzymes. DHA is synthesised by the elongation of C_{20} fatty acid precursors followed by the action of Δ^4 desaturase. Its reduced levels in the protein deficient and 0 wk group suggest reduction in the activity of this enzyme. Functionally active tissues such as brain and retina (Neuringer and Connor, 1986) are rich in DHA. It is selectively incorporated into alveolar macrophages (Huterer and Wherrett, 1985) and peritoneal macrophages (Leslie *et al.,* 1985) in rats maintained on diets enriched in fish oil or n-3 fatty acids.

The importance of long chain PUFA as eicosanoid precursors is now well recognized. Macrophages abundantly synthesize prostaglandins and leukotrienes which have potent bioregulatory roles (De Maroussem *et al.,* 1985). Prostaglandins PGE_1 and PGE_2 which are derived from n-6 fatty acid exhibit negative control in regulation of cell mediated immune response. Increase in n-3 fatty acids [eicosapentaenoic acid (EPA) and DHA] enhances the synthesis of PGE_3 (Wills, 1981) which modulates the activities of PGE_1 and PGE_2 (Leslie *et al.,* 1985; Smith *et al.,* 1985).

Table 6. Classification of macrophage fatty acids.

	Control (wk)		Protein (%)	
Fatty acid class	0	3	20	4
Saturated (C_{16}, C_{18}, C_{24})	26·8	14·1	16·0	26·6
Unsaturated ($C_{18:1}$, $C_{18:2}$, $C_{18:3}$)	16·4	10·8	9·9	16·5
Long chain PUFA eicosaenoid series ($C_{20:1}$, $C_{20:3}$, $C_{20:4}$, $C_{20:5}$)	33·0	31·0	31·6	40·6
Docosaenoid series ($C_{22:1}$, $C_{22:5}$, $C_{22:6}$)	23·7	44·9	38·7	16·5
Unsaturation index[1]	290	406	372·7	266·9
Unsaturation index[2] [Saturated]	10·82	28·83	19·6	10·04
[Unsat]/[Sat][3]	2·73	6·1	4·27	2·75
Double bond indices[4] $\frac{n6}{n3}$	0·31	0·163	0·23	0·59

The data reported above is the sum of the mean of fatty acids (% wt. of total fatty acids) represented in parentheses under each class.

The calculation of different indices was done as follows.

1. Unsaturation index (USI)= (% of unsaturated fatty acid × no. of double bonds).

2. $$\frac{\text{Unsaturation index}}{[\text{Saturated}]} = \frac{\text{USI}}{\Sigma\,\%\ \text{saturated FA}}.$$

3. $$[\text{Unsat}]\,[\text{Sat}] = \frac{\Sigma\,\%\ \text{unsaturated FA}}{\Sigma\,\text{saturated FA}}.$$

4. $$\frac{n6}{n3}\ \text{ratio} = \frac{\Sigma\,n6\ \text{FA} \times \text{double bonds}}{\Sigma\,n3\ \text{FA} \times \text{double bonds}}.$$

This regulation is essential in the prevention of pathophysiological states and may be affected in the protein deficient and 0 wk groups which contain higher n-6 fatty acids. EPA is known to be formed by the retroconversion of DHA in humans (Fischer *et al.,* 1987) and bovine endothelial cells (Hadjiagapion and Spector, 1987). Possibly in macrophages DHA may serve as an endogenous storage form of EPA.

By virtue of its disordering effect a role for DHA has also been envisaged in maintenance of membrane fluidity and associated functions (Conroy *et al.,* 1986). Observed changes in DHA levels could effect cell membrane physical properties. These parameters affect protein conformation and lipid protein interactions (Stubbs and Smith, 1984) which could reflect on the enzymes and receptor functions of macrophages. Long chain PUFA also have a role in macrophage activation and act as potent stimulators of their respiratory burst (Bromberg and Pick, 1983) and cytotoxic potential (Schlager *et al.,* 1983). Distortions in these functions have been observed in macrophages isolated from protein deficient rats (J. P. Machaiah, unpublished results).

The results presented here suggest that critical levels of macrophage lipid constituents are essential for optimum immune response. This is disturbed in protein deficient and young rats. Earlier observations have reported suboptimum

immune response in neonates (Chandra, 1984). Protein restriction has apparently down regulated the thioglycollate elicitation of macrophages to an extent comparable to that observed in young rats. Observed variations in macrophage lipid components in these groups could profoundly influence its membrane fluidity, receptor activity and eicosanoid metabolism; these parameters regulate its key immunologic functions pertaining to phagocytosis and cytotoxicity.

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