CHAPTER IV

DISCUSSION

Fat emulsions are widely accepted as condense source of energy and lone source of essential fatty acids for patients receiving intravenous total parenteral nutrition (TPN) (Carpentier, 1989). The benefits of including fat emulsion in parenteral nutrition in man are therefore well acknowledged. However, the conventional fat emulsions with egg yolk and soya as emulsifier worldwidely utilized in hospitalized patients still provide some disadvantages. Dahlan et al. (1992a) demonstrated that during 6-h intravenous infusion of fat emulsion with egg yolk lecithin as emulsifier in healthy man, red blood cell (RBC) membranes had altered in their major lipid composition, for instances cholesterol decreased while phospholipids increased. In the different study of the same investigators, Dahlan et al. (1992b) reported a depletion of omega-3 polyunsaturated fatty acids (n-3 PUFA) in RBC membranes after daily intravenous infusion of such emulsion in 5 pateints with inflammatory bowel disease for 3 months. The latter study showed an evidence that daily infusion of fat emulsions for a long period of time caused a redistribution of the PUFA between the n-6 and n-3 families. Huge presentation of n-6 PUFA in triglyceride (TG) core as well as in phospholipid (PL) surface of fat emulsions in comparison to those of n-3 PUFA could induce an accumulation of n-6 with a disappearance of n-3 PUFA's resulting in a marked depletion of n-3/n-6 ratio in circulating blood cell membranes.

Eicosapentaenoic acid (EPA) as a representative of n-3 PUFA and arachdonic acid (AA) of n-6 PUFA presented in membranes are two major precursors of prostanoids in the two series of E_2 and E_3, respectively (Simoupoulos, 1991). Recently, British Nutrition Foundation (1994) has reviewed vast literatures describing deleterious effects of alterations of prostanoid biosynthesis on biochemical changes in the body, e.g. atherogenesis, thrombogenesis, fibrinolysis etc. The alteration of

membrane lipids as well as PUFA demontrated by Dahlan et al. (1992b) as earlier mentioned might induce certain effects on membrane function (Dahlan, 1989).

Docosahexaenoate (DHA) is a major PUFA of erythrocyte membranes which prone to be impaired in various circumstances (British Nutrition Foundation, 1994). In the present experiment, we prepared a novel emulsion rich in n-3 PUFA especially DHA in PL surface aiming to utilize such an emulsion for maintaining and if possible improving n-3 PUFA status of the membranes. The exchanges of fatty acids and lipids between erythrocytes and our novel emulsion during incubation were studied in *in vitro* experiment and the results will be discussed and summarized as follows.

Fish Meal as Raw Material for Preparation of n-3 PUFA Rich Lecithin

In principle, lecithins can be obtained from all kinds of living matter, as their constituents are essential components of cell membranes (Devlin, 1993). This means that lecithins can be produced from vegetable, animal or even microbial sources. But in practical commercial terms, they are made basically from vegetable products like soybean, sunflower or rapeseed, with corn and groundnuts of minor importance. Typically, vegetable lecithins are obtained as a by product of the oil refining process, where substances containing phosphorus have to be removed to give the oil better stability and/or to facilitate further refining. Composition of typical vegetable lecithins make it obvious that there are no crucial differences between them (Pardun, 1982).

Though in the U.S. only vegetable lecithins are considered to be GRAS (generally recommended as safe), egg lecithin (historically the first lecithin to be discovered) also plays an important role, though mainly in pharmaceuticals because of their relative high price (Schneider, 1992). It is well recognized that the composition of polar lipids in egg lecithin is remarkeably different from vegetable lipids. Clear differences are also seen in the fatty acid breakdown. This fact is also confirmed in the present study as one can see in **Tables 19, 23** and **24** in previous chapter of this text.

In the present study fish meal not egg yolk or any vegetables was used as source of lecithin due to one of the main objectives of this experiment was to find

lecithins with high content of n-3 PUFA in order to utilize them as emulsifier for preparation of fat emulsion. The rich presentation of n-3 PUFA in the surface of PL of our novel fat emulsions was hypothesized in our study that it might behave as an excellent supplier of n-3 PUFA to blood cells during their circulation in blood after being intravenously infused in man. The primary task for us was to seek a good source of n-3 PUFA rich lecithin. This purpose brought fats of marine origin which had been widely known as mostly exclusive source of n-3 PUFA into our consideration. Hence, in the present study fish meal as an accessible and plentiful marine product was finally selected as raw materials for the preparation of crude lecithins.

In fact, fish meal has been used in feeds of poultry, pigs, ruminants, fish, crustaceans, pets and fur-bearing animals for long time since it increases productivity and improves feed efficiency. As good source of many nutrients, fish meal provides a unique balance of essential amino acids, energy, vitamins, minerals and trace elements, which implement other feed ingredients by correcting their deficiencies (Bimbo and Crowther, 1992). Hitherto, there are at least five supportive reasons for us to select fish meal as source of lecithin in the present study.

Firstly, fish meal has a privilege of marine animal origin which widely accepted as rich source of n-3 PUFA (Simoupoulos, 1991). Our preliminary survey showed that fish meal had prominent characteristics in its fatty acid constituents, e.g. very long chain with high unsaturation especially n-3 PUFA. Furthermore, fish meal contained lecithin in higher amounts in comparison to those obtained from fat waste of tuna canning manufactures and also contained significant proportion of n-3 PUFA's in its fatty acid profiles (Dahlan, 1996). Grade-1 fish meal called G-1 FM used in the experiment was found to contain fat as high as 13.9 % of weight with 14.3 % lecithin in its lipid composition. Fish meal lecithin prepared from our study constituted high content of n-3 PUFA especially of DHA (average of 28 and 22% in PL-FA for n-3 PUFA and DHA, respectively).

It has been known that lecithin of fish meal has an advantage of those derived from animals by containing high polar phospholipids in comparison to vegatable lecithins (Schneider, 1992). G-1 FM had 70 mole% of choline-containing

PL's with phosphatidylcholine-phosphatidylethanolamine (PE-PC) ratio of 0.18. This ratio was close to the ratio of 0.05 found in plasma of healthy men. By contrast, the conventional soya and egg yolk lecithins provided the ratios of respective 0.53 and 0.42 obviously different from PE-PC ratio previously mentioned in human plasma. Thereafter, it provides lecithin of fish meal a great advantage of human-like in comparison to other animal lecithins as well as vegetable lecithins.

Secondly, fish meal is the principal ingredients in feeds of many important species of farmed fish and shimps. It is well established that fish meal is categorized in most countries based on its protein content, with higher value going to fish meals with higher than average protein contents. In contrary to protein, fat content of fish meal is neglected and unneeded since it facilitates ease oxidation and rancidity to the products (Hardy and Masumoto, 1991). Despite of the fact that fat obtained from fish meal is a good source of arachidonic acid utilized for cat nutrition and also a rich source of n-3 PUFAs which possibly represent over 30% of the total fatty acid present, however, it has been eliminated along with fish meal manufacturing process in order to diminish origin causing rancidity and oxidation of the end product and not to invest too much for the addition of antioxidant (Bimbo, 1990). According to the Department of Fisheries and Oceans in Canada for Atlantic salmon diets and the United States federal hatcheries, fat content in fish meal was limited not exceed to 10% (Hardy and Masumoto, 1991). This figure was also defined for the limitation of fat content in fish meal used as raw material for the preparation of quality and standard animal feeds in Thailand. According to Thailand's Ministry of Agriculture and Cooperatives (TMAC), bar of fat content was ruled out from specification of fish meal raw materials in 1995. At the middle of 1995, TMAC announced the Act No. 8/1995 as a new regulation for standard of fish meals utilized for animal feed (TMAC 1995, see also Appendix). In this regard, the limitation of fish meal fat content was totally skipped out and the present Thai fish meal hence contains significant amount of fats approximately closes to its natural origin. Accordingly, fish meal was considered in our study as advantageous for this aspect.

Thirdly, apart from the advantage of new regulation as mentioned above, fish meal is also considered as a natural not processed source of fish lipids. It has been widely accepted that lipids especially PUFA well kept in its original sources especially in cell's fat vacuoles are more stable to oxidation than lipids released from their natural sources, e.g. fish oils in crude fat wastes and in fish oil stocks (Li, 1991). Therefore, fish meal as a natural source of fish lipids was utilized with its full benefits in our experiment.

Fouthly, fish meal is one of the major marine by-products upon which the aquaculture feed industry depends. Chile, Peru, Denmark, Iceland, Norway and South Africa are actually counted as the major fish meal manufacturing countries in the world and originated in 1989 approximately 97% of the world trade of fish meal (Bololanik, 1989). Paradoxically, Thailand contributes a minor part in this huge trade but aquaculture products from Thailand which known to be dependent closely and mainly to domestic fish meal production showed its export values to the world market exceeded 100,000 million bahts in 1995 thus ranked Thailand as the biggest aquaculture exporter in 1995 (Thai Farmer Bank's Research Center, 1995). Excluding fish meal, the world's aquaculture production is produced majorily in East Asia. Thai aquacultural agriculture itself especially in the area of farmed shrimp culture has been vastly expanded during this decade (Akiyama, 1991). This might explain the incredible growth rate of fish meal manufacturing industry in Thailand upto 50% during the last 5 years of 1991-1995 from 460,000 MT to 620,000 MT while the growth rate of world's fish meal production was expected to remain relatively constant during the next decade (Hardy and Masumoto, 1991; The Thai Fish Meal Producers Association, 1995, see also Appendix). Thus fish meal is plentiful and accessible for us to select unanimously as raw materials in the experiment.

Fifthly, it is believed generally that depot fats exist in cells consist largely of TG while the total lipids of various body organs and muscle tissues can by comparison contain large proportion of PL (Stansby et al., 1990). Actually, fish oil as well as crude fat waste obtained mainly from crude oil released after steaming during fish canning manufacturing process are good examples of fat from cell depot. As

earlier mentioned, our preliminary study exhibited that lecithin content in such oils, i.e. crude fat waste from tuna canning manufacturer and refined tuna oil, was much less than the content found in fish meal. This result can be explained by the fact that fish meal is produced from whole marine fish not from any released fat hence it yields high proportion of PL.

Characteristics of Lipids in Grade-1 Fish Meal

It is known that there are differences in the natural distribution of fatty acids associated with lipids such as TG and PL. For examples, it is generally believed that PL, such as lecithins and cephalins (phosphatidylethanolamine), contain more PUFA than do the TG when isolated from the same oil and tissues (Stansby et al., 1990). We also confirmed this fact in our experiment.

As mentioned earlier, fat content in Thai fish meal is exclusive factor for considering specifications of fish meal conventionally regulated in other countries. When protein is taken into account, however, specifications for Thai fish meals used in aquaculture and other animal feeds are of a similar nature to those of other countries. In general, higher protein categorizes fish meal with much higher value. In our study after selecting fish meal as raw material for the extraction of lecithin, four different grades of fish meal according to their classification were brought for studying their lipid characteristics. As indicated from their proximate nutrient values, protein content in four grades of fish meal ranged from 60-70 % (w/w) in the order of grade 4 to grade 1 whereas fat content was likely to be independent from their categories. Finally, grade 1 fish meal (G-1 FM) was chosen for further study in the experiment according to its prominent in lipid characteristics in comparison to those other three grades.

Actually, G-1 FM provided fat and lecithin in relatively higher amounts than lower grades of fish meal, G-3 and G-4 FM's (G-1 FM had 20% higher in fat and 80% in lecithin). Comparing with its rival premium grade, fat and lecithin in G-1 FM were still slightly higher than those of G-2 FM (fat contents: 13.9 vs 13.1, lecithin contents: 2.0 vs 1.6 g/100 fish meal for G-1 and G-2, respectively). Considering as

yield of lecithin obtained from the extracted crude oil, G-1 FM was likely to provide higher lecithin than G-2 FM (14.3 vs 12.5 g/100 g crude oil for G-1 and G-2 FM's, respectively). Hence, G-1 FM could provide in its content PUFA especially n-3 polyenes in much higher amount in comparison to G2-G4 FM's (495 vs 355, 288 and 232 mg PUFA/100 g fish meals and 410 vs 302, 256 and 204 mg n-3 PUFA for G-1, G-2, G-3 and G-4 FM's, respectively). Focussing into individual n-3 PUFA, G-1 FM also yielded DHA at the level of 324 mg/100 g fish meal which was obviously higher than the values of 235, 183 and 150 for DHA yielded from G-2, G-3 and G-4 FM's, respectively. In summary, G-1 FM was found as a rich source of lecithin with high n-3 PUFA especially DHA in their PL moiety.

Extraction of Fish Meal Lecithin

Lecithins in fish meal are polar lipids majorily present as cell membrane constituents. They are co-extracted with TG by hexane extraction. Since fish meal lecithin like other animal lecithins are different from vegetable lecithins in that they are not obtained as by-products of TG production, it is therefore impossible to extract PL or lecithin from solid fish meal by hexane treatment only. Binding of lecithins to proteins as well as to carbohydrates is so tight that more polar lipids such as alcohols must be used to disrupt links between PL-protein or PL-carbohydrate. Thus a favorable method of obtaining crude fish meal lecithin is to extract powdered fish meal with alcohol, e.g. methanol or ethanol (Schneider, 1992).

In our experiment, the advantage of pretreatment samples with alcohol was verified. Extraction of fish meal by n-hexane alone without alcohol pretreatment yielded fat and lecithin contents of 6.9 and 0.53 g/100 g fish meal, respectively. A step of alcohol treatment of sample prior to extraction of crude fats with n-hexane added up the yields of fat and lecithin contents upto 11.4-11.5 and 0.97-1.12 g/100 g fish meal, or increased 65-67% and 83-112%, respectively. In summary, alcohol pretreatment before conventional crude fat extraction gains 1.7 times productions of

fat and twice productions of lecithin. Polar solvent such as alcohol extracted more polar lipids resulting in their higher proportion in the end production.

Comparing between the advantages of methanol and ethanol for summing up yields of fat and lecithin productions, there was no evidence demonstrating in the present study that both alcohols provided different benefit for this purpose. Productions of fat and lecithin obtained from the extraction procedure with either methanol and ethanol pretreatment were relatively similar. However, the superiority of higher polar solvent like methanol was observed after deoiling process with acetone. Lecithin found in dried acetone insoluble extract (polar lipid fraction) of sample pretreated with methanol was significantly higher than that pretreated with ethanol (23.80±0.50 vs 21.80±1.28 g/100g extracts for methanol and ethanol pretreatments, respectively). Pretreatment with methanol thus provided approximately 8% higher yield than that of ethanol. This might be a benefit of trace higher polar solvent like methanol presented in acetone during deoil process. More polar solvent presented in the mixture might induce more lecithin released into polar acetone insoluble fraction.

Since crude oil, extracted from fish meal utilizing alcohol-n-hexane as extraction solvent system, contains significant amount of TG, deoiling is the most important in treatment of crude oil derived from fish meal in order to obtain lecithin. Simple in principle, it is based on the fact that polar lipids such as lecithin in contrast to neutral lipids are almost insoluble in acetone. Thus acetone is the solvent of choice in this experiment. In principle, acetone removes first the water then TG of crude fish meal oil, leaving acetone insoluble PL. Additionally, acetone manifests the superiority of methanol over ethanol for pretreating sample during the extraction of polar lipids such as lecithin as earlier explained.

According to the American's Oil Association of Chemists (AOAC), acetone extraction method was recommended for determination of the oil content of fish meal. This is a two-step procedure involving initial extraction with acetone, then an acid hydrolysis with 4 N hydrochloric acid followed by a second acetone extraction. This type of procedure is needed because as most fish meals are stored after

manufacturing, their lipid gradually reacts with the protein to form compounds that cannot be extracted until after an acid hydrolysis is performed. In such cases a fish meal with an initial lipid content of 12% may, after prolonged storage and using a single extraction, appear to have a lipid content of 2% or less (Standsby, 1990).

In the present study, however, acid hydrolysis was not applied in the extraction procedure. It can be explained by the following answers. Firstly, fish meal was supplied fresh from manufacturer to Department of Marine Sciences before transferring to FORC. Secondly, pretreatment fish meal with alcohol was proven to be sufficient for releasing total fat, the values of fat content obtained from our extraction were not different from those analyzed in fresh sample by manufacturers.

Regarding fatty acids in the obtained fish meal lecithins prepared in our experiment, they distributed into three groups, i.e. saturated, monenes and polyenes, in 43.34, 20.08 and 29.47 % of total fatty acids, respectively. Among polyenoic fatty acids, DHA contributed in 17.51% whereas EPA joined 6.42%. Noticeably, DHA in lecithin was much higher than that of neutral lipid or TG (17.51 vs 14.89). The nature and distribution of fatty acids in lipids extracted from fish are described elsewhere (Stansby et al., 1990). In conclusion, lecithins extracted from our experiment, despite in amounts considerably less than those of TG, was a rich source of n-3 PUFA especially DHA. Among the various PL that found in fish meal, phosphatidylcholine (lecithins) occurs to the greatest extent (50.6%) with sphingomyelin being the second most common class (17.98%). The content of n-3 PUFA could be raised in significant proportion by means of polar solvent extraction as demonstrated earlier or described elsewhere (Dijkstra and Van Opstal, 1989; Benjakul and Taylor, 1994; Wantwin et al., 1995), as well as by enzymatic treatment (Maehr et al., 1994; Yamane et al., 1992). Maehr et al. (1994) demonstrated that the utilization of Amano P lipase enzyme could convert n-3 PUFA content in fat emulsion lipids in glycerol solution from 29-34% to approximately 50%. Yamane et al. (1992) employed the technique of lipase-catalyzed acidosis without solvent to increase the proportion of DHA/EPA. Hence the manipulation of PUFA in fat emulsion quantitatively and qualitatively are possible in future experiment.

Concerning to lecithins, it was found in the experiment that PL subclasses of the obtained lecithin were not purturbed by the procedure of three consecutive organic solvent extraction. This phenomenon could be explained by the observation of Smiles et al. (1989). In their experiment, acetone insoluble extracted from various vegetable oils using six different degumming reagents were separated into PL components. They found that the degumming processes did not dramatically alter the PL profiles.

Considering into the role of PL in metabolism of emulsion, it was found that PL subclasses of lecithin utilized as emulsifier for preparing fat emulsions especially in term of the ratio of PE/PC might influence the intravascular metabolism of fat emulsions (Richelle et al., 1988). Lecithin of fish meal prepared in the present experiment had PE/PC ratio of 0.18 approximately close to that of human plasma (0.05). Noticeably, PE/PC ratio of two lecithins, egg yolk and soya, conventionally utilized as emulsifier for preparing commercial fat emulsions was obviously different from that of plasma (0.42 and 0.53 vs 0.05 for egg yolk, soya and plasma, respectively). The similarity of PE/PC ratio of fish meal lecithin to plasma lecithin might provide benefit to the metabolism of fat emulsion particles emulsified by fish meal lecithin during their circulation in the blood stream. However, this speculation has to be clarified in different experiment.

Preparations of Fat Emulsions

It has been widely recognized that fat intake either orally (Diboune et al., 1992; Hoffman et al., 1993; Leaf et al , 1995), or enterally (Adams et al., 1993), or intravenously (Dahlan et al., 1992b; Abushufa et al., 1995) could induce alteration of fatty acids in PL of blood cells and other tissues. Furthermore, it has been demonstrated that the ratio of PL to TG of fat emulsion influnces the changes in fatty acids of membrane PL (Carpentier, 1989; Dahlan et al, 1992a; Roulet et al, 1993). In general, fat emulsions are essentially composed of TG and PL and are used for supplying energy to various tissues (Carpentier et al., 1986).

Carpentier (1989) described the existence of PL liposomes in fat emulsion bottle. In his explanation, there were actually two types of particles which were present in lipid emulsion, TG rich particles and PL rich particles. Both types of particles play important but different roles in lipid metabolism in the body after they are intravenously in fused into blood circulation. Since fat intake influences fatty acid composition of tissues as earlier mentioned, then the difference in composition of fatty acids between body tissues and fat emulsions would induce the exchanges and transfer between those fatty acids. Hence fat emulsions is no longer considered only as a means for delivering energy to different tissues of the body but also as a tool for better understanding and for interacting with the metabolism of endogenous lipoproteins and plasma transport of cholesterol. The development of new fat emulsions requires an integration of updated knowledge in all various disciplines.

In the present study, we prepared a novel fat emulsion with high proportion of PL thus yielded much higher presentation of PL liposomes in the solution. Furthermore, this prepared fat emulsion demonstrated in their PL the significant proportion of n-3 PUFA which made them different from conventional fat emulsions. The aim of this preparation was to employ the prepared emulsion for suppressing the loss of n-3 PUFA from circulating blood cells. Three different emulsions with high PL to TG ratio of 1:3, w/w, were prepared by utilizing three different lecithins obtained from either fish meal, egg yolk or soya, as emulsifier. Fish meal emulsion was prepared according to the objective of the study whereas two other emulsions were utilized purposing for the comparison.

In fact, it was not only PL surface of three fat emulsions were different but the composition of TG in the emulsions' core were different as well. Fish meal derived lecithin-rich fat emulsion (FM-LRFE) had its core derived from TG obtained totally from fish meal. EY-LRFE or egg yolk lecithin emulsion had its core derived from TG of two different sources, i.e. egg yolk and soya at the ratio of 1:1, w/w. SY-LRFE or soya lecithin emulsion had its core wholely derived from TG of soya. TG in the core might have some influence on fatty acid exchanges between emulsion and RBC during their incubation. However, the results of net changes as described later

considered the effect of lipid composition in the whole particles without any exclusion of either TG core or PL surface.

Focusing into fatty acids in the surface of all prepared emulsions, It was found that PL surface and liposomes of FM-LRFE constituted high content of DHA as high as 22.16% whereas SY-LRFE constituted hugh content of linoleic acid at the level of 63.8 g/100 g PL-FA. The extreme amounts of DHA in FM-LRFE and LA in SY-LRFE were thus speculated for their influence on erythrocyte fatty acids after their interaction or attachment. In this respect, Dahlan et al. (1992b) demonstrated in their investigation that fatty acids in both TG and PL of emulsions influenced the alteration of fatty acids in erythrocyte membranes.

Effects of Fat Emulsions on the Exchanges of Lipids and Fatty Acids in Red Blood Cells

Recent study of Sadou et al. (1995) on the incorporation of DHA and EPA from dietary TG into plasma lipoproteins provided some interesting prospects for understanding the future application of lecithin liposomes demonstrating in the present study. The authors investigated how the distribution of EPA and DHA in the sn-2 and sn-1 (3) of fish oil TG influenced their respective incorporation into TG and PL of lipoproteins. The results strongly suggested that the higher TG incorporation of DHA and the higher metabolic availability of EPA for PL accumulation depended on their respective preferential sn-2/sn-1 (3) positions in fish oil TG structure. Sadou et al. (1995) confirmed many previous investigations that the presentation of n-3 PUFA's on TG and PL of lipoproteins was strictly controlled by dietary fats (Galli et al., 1981; Field et al., 1985). Therefore, the alteration of n-3 PUFA composition in blood cells membranes via the interaction and exchange of those fatty acids with plasma lipoproteins is gradual, slow and limited (Dahlan, 1989; Owen et al., 1984). We demonstrated in the present experiment how to accelerate this exchange of fatty acids in membranes.

It is well established that the mature erythrocyte is incapable of direct PL or cholesterol synthesis, and cannot desaturate or elongate its fatty acid constituents (Shohet, 1970). The erythrocyte membrane lipid composition is continuously and gradually remodeled during the cell lifetime by exchanging the coated lipid with plasma lipids (Jain and Shohet, 1982). Menbrane PLs and their fatty acids, are also renewed by several mechanism as described elsewhere (Dahlan, 1989). Membrane fatty acids are turned over either by deacylation-reacylation in situ where plasma albumin-FFA complexes play a crucial roles as fatty acid donor and acceptor. Other mechanisms are the renewal of membrane PLs by acylation and incorporation of plasma lysophospholipids, and by passive exchange or net transfer of PL and cholesterol with lipoproteins. Additionally, both the content and composition of membrane PL may be alterd by the incorporation or fusion of exogenous PL as demonstrated for erythrocyte membranes during intravenous infusion of Intralipid emulsion. Hence, we employed RBC as a suitable model for our observation of lipid and fatty acid transfer from our prepared fat emulsions and liposomes.

During the incubation with three fat emulsions/liposomes in our experiments, no alteration was not observed for the major membrane lipids either cholesterol or PL. The ratio between those two major lipids was maintained during the incubation with various concentrations of all three emulsions. However, the alterations of erythrocyte membrane fatty acid composition were observed in different degree depending on the types of the incubated emulsions. FM-LRFE markedly rose membrane fatty acids in two groups: monoenes and polyenes especially DHA. The significant correlation with r^2 value as high as 0.87 was found between DHA in membrane PL and the concentration of emulsion/liposome PL. It was likely that DHA from FM-LRFE replaced PUFA in n-6 family, i.e. LA and AA, due to the decrement of both mentioned n-6 PUFA were found in the similar but opposite degree of DHA increment.

During the incubation with EY-LRFE, no drastic alteration was found in any fatty acids of the RBC membranes. However, significant rise was observed in

oleic acid of RBC from 13.33 to 13.96% with r^2 value between membrane monoene and PL concentration of 0.91 as high. This might be expalined by half the content of oleic acid in membrane (13.33%) in comparison to that in PL of EY-LRFE (28.7%). Apart from monoenes, DHA of membrane also dropped from 5.19 to 4.57 in EY-LRFE group corresponding to the simultaneous rises of membrane saturated and monounsaturated fatty acids in similar incubation.

Incubation with SY-LRFE showed significant rise of membrane LA due to the huge presentation of this fatty acid in emulsion's PL. This marked increment of LA in membranes during the incubation with SY-LRFE purturbed many PUFA especially it was likely that it replaced AA as well as DHA of the membranes which might lead to the impairment of certain membrane functions. It is widely accepted that PL as major membrane lipid composition and AA as major n-6 PUFA in PL moiety play crucial roles in membrane function. The PL/AA ratio is indicated as a second messenger system in signaling the regulation of hormone and local mediator releases. In vivo and in vitro evidence indicates that these effector activate phospholytic enzymes in cellular membranes (Hartl and Wolfe, 1990). In addition, the products of these enzymatic reactions can serve as second messengers that can potentially influence glucose, lipid and protein metabolism at the cellular level. Alterations in this second messenger system could be involved in metabolic changes asscociated with some pathologic conditions as well as certain drug treatment.

Therefore, it is evidenced that any alteration of membrane PUFA in consequent to the prologed (and short term) incubation of blood cells with fat emulsions intravenously infused into bloodstream more or less might affect membrane functions. The present study exhibited the highest alteration of membrane incubated with SY-LRFE in term of n-6 PUFA and implied that this deleterious effect might be converted by balancing PL of fat emulsion's emulsifier by the appropriate ratio of lecithins derived from fish meal with other conventional lecithins. However, the effects of FM-LRFE as well as n-3 PUFA rich liposomes obtained from fish meal for their on either the correction of depleted membrane n-6 PUFA, or alteration of mediators and prostanoids are strongly recommended for further studied. A better understanding of

the metabolism of PUFA in membranes and their manipulation of composition with liposomes could reveal new possibilities for future therapeutic interventions.