

การเปลี่ยนแปลงองค์ประกอบกรดไขมันของเกล็ดเลือดของมนุษย์โดยอิมัลชันไขมันที่มี  
มีเลซิทีนสูงหลายชนิดซึ่งมีแกนของไตรอะซิลกลีเซอรอลและผิวของฟอสโฟลิปิดต่างกัน

นางสาวสุพัตริตรา ชาญประเสริฐ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต

สาขาวิชาเทคโนโลยีทางชีวภาพ

บัณฑิตวิทยาลัย จุฬาลงกรณ์มหาวิทยาลัย

ปีการศึกษา 2540

ISBN 974-638-912-2

ลิขสิทธิ์ของบัณฑิตวิทยาลัย จุฬาลงกรณ์มหาวิทยาลัย

**THE MODIFICATION OF HUMAN PLATELET FATTY ACID  
COMPOSITION INDUCED BY LECITHIN-RICH FAT EMULSIONS  
WITH DIFFERENT TRIACYLGLYCEROL CORES AND  
PHOSPHOLIPID SURFACES**

**Miss Supantitra Chanprasert**

**A Thesis Submitted in Partial Fulfilment of the Requirements  
for the Degree of Master of Science in Biotechnology**

**Program of Biotechnology**

**Graduate School**

**Chulalongkorn University**

**Academic Year 1997**

**ISBN 974-638-912-2**

Thesis Title            The modification of human platelet fatty acid composition induced by lecithin-rich fat emulsions with different triacylglycerol cores and phospholipid surfaces

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## C827254 : MAJOR BIOTECHNOLOGY

KEY WORD: PLATELET/FISH MEAL/LECITHIN/EMULSION/ OMEGA-3

SUPANTITRA CHANPRASERT: THE MODIFICATION OF HUMAN PLATELET FATTY ACID COMPOSITION INDUCED BY LECITHIN-RICH FAT EMULSIONS WITH DIFFERENT TRIACYLGLYCEROL CORES AND PHOSPHOLIPID SURFACES.

THESIS ADVISOR: ASSOC. PROF. WINAI DAHLAN , Ph.D., THESIS CO-ADVISOR : ASSOC. PROF. WICHAI CHERDSHEWASART , Ph.D. 205 pp. ISBN 974-638-912-2

Platelets are the major cells that produce eicosanoids such as prostaglandin (PG) from membranes unsaturated fatty acids (PUFA): EPA and arachidonic acid (AA). AA-derived PGs are provasoconstrictory and proaggregatory whereas EPA-derived PGs yields the opposite properties. EPA and other n-3 PUFA; DHA, replace AA in membranes and turn cell to be antiatherogenic and antithrombogenic which consequently prevent the body from stroke and cardiovascular disease. In our previous experiment, we designed lecithin-rich fat emulsions (LRFE) with high proportion of DHA in phospholipid moiety. This novel emulsion was able to supply n-3 PUFA effectively to red blood cells. The purpose of the present study was whether our four LRFE's with lecithins derived from fish meal (FM-LRFE), soya (SY-LRFE), soya cored with fish oil (SL-FOFE) and egg yolk which was commercially available (20% Lipofundin), restructured n-3/n-6 PUFA ratio of platelet membranes.

Platelet concentrates (PC) were prepared and provided for the experiment from Thai Red Cross. They were incubated for 1 h at 22 °C with each freshly prepared LRFE at the phospholipid (PL) concentrations of 0, 100, 300, 600 mg/dl incubation mixture and platelet concentration of approximately  $1.86 \times 10^9$  cells/ml. The increased ratio of n-3/n-6 PUFA of platelets after incubation with FM-LRFE at 600 mg PL/dl autologous plasma was 1.8 times lower than the mixture with plasma free. In addition, all altered fatty acids on platelets were maintained in plasma-free condition for at least 5 h. Incubation with FM-LRFE, the increase of n-3/n-6 PUFA ratio was dose dependent ( $Y=0.16+3E-04 X$ ,  $p<0.001$ ). The ratio elevation appeared with much less extent under incubation condition with SY-LRFE ( $Y=0.143+3E-05X$ ,  $p<0.005$ ). The latter showed EPA and DHA decreased whereas major n-3 PUFA, alpha-linolenate (ALA), increased. Incubation with SL-FOFE demonstrated that n-3 PUFA in emulsion's core provided trace effect to the fatty acid exchanges occurred on the surface. The 20% Lipofundin gave the least alteration of platelet fatty acids.

In conclusion: platelet membranes' PUFA profiles is able to be restructured as needed. FM-LRFE supplies n-3 PUFA whereas SY-LRFE provides n-6 PUFA to the platelet membranes. The higher n-3/n-6 PUFA ratio of platelet membranes is benefit for the production of antithrombogenic PG.

ภาควิชา.....

สาขาวิชา..... เทคโนโลยีทางชีวภาพ

ปีการศึกษา..... 2540

ลายมือชื่อนิติกร..... สุกนิจชา ชานพราเสร์

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ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....

สุพัตริตรา ชาญประเสริฐ : การเปลี่ยนแปลงองค์ประกอบกรดไขมันของเกล็ดเลือดของมนุษย์โดยอิมัลชันไขมันที่มีเลซิทีนสูงหลายชนิดซึ่งมีแกนของไตรอะซิลกลีเซอรอลและผิวของฟอสโฟลิปิดต่างกัน ( THE MODIFICATION OF HUMAN PLATELET FATTY ACID COMPOSITION INDUCED BY LECITHIN - RICH FAT EMULSIONS WITH DIFFERENT TRIACYLGLYCEROL CORES AND PHOSPHOLIPID SURFACES ) อาจารย์ที่ปรึกษา : รศ.ดร.วินัย ณะห์ลัน, อาจารย์ที่ปรึกษาร่วม : รศ.ดร. วิชัย เชิดชูวิทยาศาสตร์, 205 หน้า. ISBN 974-638-912-2

เกล็ดเลือดเป็นเซลล์หลักที่ผลิตสารไอโคซานอยด์ เช่น พรอสตาแกลนดินจากกรดไขมันชนิดไม่อิ่มตัวสูง คือ กรดไอโคซะเพนตะอีโนอิก (EPA) และกรดอะริชโคนิก (AA) พรอสตาแกลนดินที่สร้างจาก AA เกี่ยวข้องกับการหดตัวของหลอดเลือดและการจับกลุ่มกันของเกล็ดเลือดในขณะที่พรอสตาแกลนดินจากEPA มีคุณสมบัติตรงกันข้าม EPAและกรดไขมันไม่อิ่มตัวสูงกลุ่มโอเมก้าสาม (n-3 PUFA) เช่นโคโคสะเฮกซะอีโนอิก (DHA) จะเข้าไปแทนที่ AA ในเมมเบรนและเปลี่ยนการทำงานของเซลล์ให้ยับยั้งการจับตัวสะสมกันของไขมันตามผนังหลอดเลือดและการรวมตัวกันเกิดลิ่มเลือดซึ่งจะช่วยป้องกันการเกิดการอุดตันอย่างเฉียบพลันของหลอดเลือดในสมอง (stroke) และโรคหัวใจและหลอดเลือด ในการศึกษาก่อนหน้านี้ได้มีการออกแบบอิมัลชันไขมันที่มีเลซิทีนสูง (LRFE) ซึ่งมีสัดส่วนของ DHAสูงในส่วนของฟอสโฟลิปิด อิมัลชันไขมันชนิดใหม่นี้สามารถจ่าย n-3 PUFA ให้กับเม็ดเลือดแดงได้อย่างมีประสิทธิภาพ จุดมุ่งหมายของการวิจัยครั้งนี้เพื่อศึกษาถึงผลของอิมัลชันไขมัน 4 ชนิดซึ่งเตรียมจากเลซิทีนจากปลาป่น (FM-LRFE) ถั่วเหลือง (SY-LRFE) ถั่วเหลืองที่มีแกนเป็นน้ำมันปลา (SL-FOFE) และเลซิทีนจากไข่แดงของไข่ไก่ (20%Lipofundin) ต่อการเปลี่ยนแปลงสัดส่วนของ n-3/n-6 PUFA บนเมมเบรนของเกล็ดเลือด

นำเกล็ดเลือดเข้มข้นที่มีจำนวนเซลล์  $1.86 \times 10^9$  เซลล์ต่อมิลลิลิตรซึ่งได้รับจากสภากาชาดไทยมาทำการทดลองร่วมกับอิมัลชันไขมันที่มีเลซิทีนสูงที่ความเข้มข้นของฟอสโฟลิปิด 0, 100, 300 และ 600 มิลลิกรัมต่อเซลล์เป็นเวลา 1 ชั่วโมงที่ 22 องศาเซลเซียส สัดส่วนที่เพิ่มขึ้นของ n-3/n-6 PUFA ของเกล็ดเลือดหลังจากแช่กับ FM-LRFE ที่ 600 มิลลิกรัมฟอสโฟลิปิดต่อเซลล์ในภาวะที่มีพลาสมาที่มีค่าต่ำกว่าภาวะที่ไม่มีพลาสมาประมาณ 1.8 เท่า นอกจากนี้ยังพบว่ากรดไขมันที่เปลี่ยนแปลงบนเกล็ดเลือดในภาวะไม่มีพลาสมาสามารถคงสภาพอยู่ได้ไม่น้อยกว่า 5 ชั่วโมง การแช่กับ FM-LRFE สัดส่วนของ n-3/n-6 PUFAจะเพิ่มขึ้นตามความเข้มข้นของอิมัลชันดังสมการ  $Y = 0.16 + 3E-04X$ ,  $p < 0.001$  ในขณะที่การแช่กับ SY-LRFE การเพิ่มขึ้นของ n-3/n-6 PUFA ตามความเข้มข้นของอิมัลชันจะมีค่าน้อยกว่าตามสมการ  $Y = 0.143 + 3E-05X$ ,  $p < 0.005$  ซึ่งจากผลสรุปแสดงให้เห็นว่า EPA และ DHA มีการลดลง ในขณะที่มีการเพิ่มขึ้นของกรดอัลฟาไลโนลินิก (ALA) ซึ่งเป็นกรดไขมันหลักของกรดไขมันกลุ่มโอเมก้าสาม การแช่กับ SL-FOFE แสดงให้เห็นว่ากรดไขมันกลุ่มโอเมก้าสามในส่วนแกนของอิมัลชันมีผลเพียงเล็กน้อยต่อการเปลี่ยนแปลงของกรดไขมันบนผิวของเกล็ดเลือด สำหรับการแช่ใน 20% Lipofundin นั้นพบว่ามีการเปลี่ยนแปลงของกรดไขมันน้อยที่สุด

การศึกษานี้สามารถปรับเปลี่ยนองค์ประกอบของ PUFA บนเมมเบรนของเกล็ดเลือดตามที่ต้องการได้ FM-LRFE สามารถจ่าย n-3 PUFA ในขณะที่ SY-LRFE ให้ n-6 PUFA แก่เมมเบรน สัดส่วนของ n-3/n-6 PUFA ที่เพิ่มขึ้นบนเมมเบรนของเกล็ดเลือดนั้นมีประโยชน์ต่อการสร้างสารพรอสตาแกลนดินซึ่งมีผลยับยั้งการเกิดลิ่มเลือดอันเป็นผลดีต่อการป้องกันโรคหัวใจและหลอดเลือด

ภาควิชา .....  
สาขาวิชา ..... เทคโนโลยีทางชีวภาพ .....  
ปีการศึกษา ..... 2540 .....

ลายมือชื่อนิติ ..... สุพัตริตรา ชาญประเสริฐ  
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ลายมือชื่ออาจารย์ที่ปรึกษาร่วม .....

## ACKNOWLEDGEMENTS

I would like to express my deepest sincere gratitude to my advisor, Associate Professor Dr. Winai Dahlan, for his meaningful supervision, creative guidance and encouragement which enable me to carry out my study successfully throughout this thesis.

My special appreciation is expressed to my co-advisor, Associate Professor Dr. Wichai Cherdchewasart, for his valuable suggestions, full encouragement and kindness throughout this study.

My sincere gratitude is also extended to Assistant Professor Dr. Byaporn na Nagara, Associate Professor Dr. Somkiat Piyatiratitivorakul and Associate Professor Surat Komindr for serving as thesis committee, for their valuable comments and also useful suggestions.

I am indebted to Mrs. Aroonrat Chantanakajornfung, the Chief of Plasma and Fractionation Section for her meaningful supervision and kindness and all members of her Section in the National Blood Center, Thai Red Cross Society for their assistance and friendship.

My acknowledgement is also expressed to The Fats and Oils Research Center ( FORC ) for chemicals and instruments supported throughout this thesis and I would like to thank the Departments of Clinical Microscopy and Clinical Chemistry , Faculty of Allied Health Sciences, Chulalongkorn University for the access to use some necessary instruments for my thesis.

I am also grateful for the financial support of the National Research Council of Thailand and FORC during my study.

Special thanks are also expressed to all the friends of the Biotechnology Programme and the members of the FORC for their sincerity, assistance and friendship.

Finally, I would like to express my gratitude and deepest appreciation to my father, to the memory of my mother and to members in my family for their infinite love, great attention, understanding and encouragement which never be forgotten.

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**ABBREVIATIONS**

AA	=	arachidonic fatty acid (C 20:4 n-6)
ALA	=	alpha- linolenic acid (C 18:3 n-3)
AOAC	=	American' s Oil Association of Chemists
BHT	=	2,6-di-tert-butyl-4-methylphenol
BNF	=	British Nutrition Foundation
CAD	=	coronary artery disease
CHD	=	coronary heart disease
CPD	=	citrate phosphate dextrose
CVD	=	cardiovascular disease
DHA	=	docosahexaenoic acid (C22:6 n-3)
dl	=	decilitre (100 ml)
EDRF	=	endothelial-derived relaxing factor
EPA	=	eicosapentaenoic acid (C20:5 n-3)
EY-LRFE	=	egg yolk-derived lecithin-rich fat emulsion
FA	=	fatty acid
FAMEs	=	fatty acid methyl esters
FFP	=	fresh frozen plasma
FM	=	fish meal
FM-LRFE	=	fish meal-derived lecithin-rich fat emulsion

FORC	=	Fats and Oils Research Center, Chulalongkorn University
g	=	gram
G-1 FM	=	grade 1 fish meal
GRAS	=	generally recommended as safe
h	=	hour
HCT	=	hematocrit
HDL	=	high density lipoprotein
IS	=	internal standard
LA	=	linoleic acid
LCT	=	long-chain triglyceride or triacylglycerol
LDL	=	low density lipoprotein
LE	=	lecithin
LT	=	leucotriene
MCT	=	medium-chain triglyceride or triacylglycerol
mg	=	milligram
min	=	minute
ml	=	millilitre
μl	=	microlitre
MUFA	=	monounsaturated fatty acid
n-3,ω-3	=	omega 3
n-6,ω-6	=	omega 6
°C	=	degree Celcius

PAF	=	platelet- activating- factor
PC	=	platelet concentrate
PhC	=	phosphatidylcholine
PE	=	phosphatidylethanolamine
PG	=	prostaglandin
PI	=	phosphatidylinositol
PL	=	phospholipids
PL-FA	=	phospholipid fatty acids
PLT	=	platelets
PPP	=	platelet-poor plasma
PRC	=	packed red cell
PRP	=	platelet-rich plasma
PUFA	=	polyunsaturated fatty acid
$r$	=	coefficient of correlation
$r^2$	=	coefficient of determination
RBC	=	red blood cell or erythrocytes
S.D.	=	standard deviation
SAFA	=	saturated fatty acid
sec	=	second
SL-FOFE	=	soya-lecithin fish oil mixed fat emulsion
SY-LRFE	=	soya-derived lecithin-rich fat emulsion
TG	=	triacylglycerols or triglycerides
TG-FA	=	triglyceride fatty acids

TMAC	=	Thailand's Ministry of Agriculture and Cooperatives
TPN	=	total parenteral nutrition
TX	=	thromboxane
VLDL	=	very low density lipoprotein
WB	=	whole blood
WBC	=	white blood cell