REGULATION OF PHOSPHOLIPID REMODELLING BY THYROID HORMONES IN THE RAT HEART

by
Vernon Wayne Dolinsky

A thesis submitted to the Faculty of Graduate Studies
The University of Manitoba

In partial fulfillment of the requirements for the degree
Master of Science

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VERNON WAYNE DOLINSKY

A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of Manitoba in partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE

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DEDICATION

To Mom and Dad

Thank you for your constant encouragement and support
through the long hard years of work.
Without it I could never have made it to this point.

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I owe a huge debt to my supervisor Dr. Grant M. Hatch. Thank you Grant, for giving me the opportunity to do scientific research, I have learned much from your fine example, how to question the world around us and then seek the answers. Without your constructive criticisms and constant encouragement this work would not have been possible. It is with sadness that I have switched supervisors, but I will always carry your lessons with me.

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To Rick, Sonny, Chris and the rest of the Chown building gang: Keep on Rockin'

To Doug, Mariko and the rest of the Biochem gang: Stay out of trouble or you may find ice in your bed.

To Emma, thanks for coffee and the laughs.

There are not many joys in life comparable to a discovery.

After a long period of patient research,

What once seemed so chaotic and contradictory,

Comes out of a wild confusion of facts,

A picture emerging from the mist,

Glittering under the rays of the sun,

The truth stands in all its beauty and might.

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ABBREVIATIONS

AMP	Adenosine-5'-monophosphate
ATP	Adenosine-5'-triphosphate
BHK	Baby hamster kidney
Ca^{2+}	Calcium
CO_2	Carbon dioxide
CL	Cardiolipin
CoA	Coenzyme A
cDNA	Complementary deoxyribonucleic acid
CMP	Cytidine-5'-monophosphate
CDP	Cytidine-5'-diphosphate
CTP	Cytidine-5'-triphosphate
Da	Dalton
DG	1,2-diacyl-*sn*-glycerol
DLCL	Dilysocardiolipin
dpm	disintegrations per minute
g	gram
L	litre
LPC	Lysophosphatidylcholine
LPC AT	Acyl-Coenzyme A:1-acyl-glycerophosphocholine acyltransferase

LPE	Lysophosphatidylethanolamine
LPE AT	Acyl-Coenzyme A:1-acylglycerophosphoethanolamine acyltransferase
M	Molarity of solution
MAP	Mitogen activated protein kinase
MLCL	Monolysocardiolipin
nmole	nanomole
PA	Phosphatidic acid
PC	Phosphatidylcholine
PE	Phosphatidylethanolamine
PG	Phosphatidylglycerol
PI	Phosphatidylinositol
PS	Phosphatidylserine
P_i	Phosphate
PP_i	Inorganic Phosphate
pH	Powers of Hydrogen
PTU	6-*n*-propyl-2-thiouracil
T_3	3,5,3'-triiodothyronine
T_4	L-thyroxine
TG	Triacylglycerol
Tris	Trizma base
µ	micro

ABSTRACT

Membrane bound phospholipids require a distinct fatty acid composition. This is achieved through the deacylation-reacylation cycle that involves the breakdown of the parent phospholipid to a fatty acid and its lysophospholipid derivative by a phospholipase A activity. Subsequently, phospholipids are resynthesized through the action of an acyltransferase that attaches acyl-Coenzyme A thioesters to the lysophospholipid. This cycle must be carefully regulated so that the correct fatty acid composition of the phospholipids are maintained, otherwise the normal functioning of the membrane and membrane bound enzymes could be jeopardized. Thyroid hormones have profound effects on the heart. Since it is established that thyroid hormones regulate many lipid metabolizing enzymes, it is possible that the deacylation-reacylation cycle of certain phospholipids may also be regulated by thyroid hormones.

The effect of a thyroid condition on phospholipid fatty acid metabolism was examined in isolated rat hearts perfused in Langendorff mode for 30 min with [1-^{14}C]oleic acid (18:1) bound to albumin in a 1:1 molar ratio. A 1.5 fold ($P < 0.025$) increase in the formation of [^{14}C]phosphatidylethanolamine was observed in hearts from hyperthyroid animals compared to controls (data expressed as dpm/g heart). No statistically significant increases in the formation of other [^{14}C]phospholipids were observed in the hearts of hyperthyroid animals compared to controls. Similar increases ($P < 0.025$) in the formation of [^{14}C] phosphatidylethanolamine were observed in hearts from hyperthyroid animals compared to controls, that were perfused with [1-^{14}C]palmitic acid (16:0) and [1-^{14}C] linoleic acid (18:2). However when perfused with [1-^{14}C]arachidonic acid, a decrease in the formation of [^{14}C] phosphatidylethanolamine was observed in hearts from

hyperthyroid animals compared to controls. Hypothyroidism did not significantly affect the formation of [^{14}C]phosphatidylethanolamine from [1-^{14}C]palmitic (16:0), oleic (18:1) and linoleic (18:2) acids. However, the formation of cardiac [^{14}C] phosphatidylethanolamine from [1-^{14}C]arachidonic acid (20:4) was decreased by 57% ($P < 0.025$) in hypothyroid animals compared with controls.

Since the most dramatic effects of thyroid condition in the perfused rat heart were directed towards phosphatidylethanolamine, the *de novo* biosynthetic pathways of phosphatidylethanolamine were examined in hearts perfused for 30 min with [1,2-^{14}C]ethanolamine or [^{3}H(G)]serine. The thyroid condition had no significant effects on either the CDP-ethanolamine pathway or the decarboxylation of phosphatidylserine to phosphatidylethanolamine in the perfused rat heart. In addition, the thyroid condition had little effect on the pool size of phosphatidylethanolamine in the heart (expressed as nmoles/g heart) or the other cardiac phospholipids.

These results suggested that the thyroid condition affects the remodeling of phosphatidylethanolamine in the heart. When the fatty acid molecular composition of phosphatidylethanolamine in the heart was examined, a significant 47% increase ($P < 0.025$) increase in the oleic acid content of hyperthyroid animals compared to controls was observed. Hypothyroid animals had a 30% ($P < 0.025$) increase in the palmitic acid content of phosphatidylethanolamine in the heart compared to controls. The increased oleic acid (18:1) content of phosphatidylethanolamine in hearts from hyperthyroid animals was likely a consequence of the 74% increase ($P < 0.025$) in the activity of microsomal acyl-CoA:1-acylglycerophosphoethanolamine acyltransferase (expressed as nmole/min/mg protein). The increased palmitic acid (16:0) content of phosphatidylethanolamine in hearts from hypothyroid animals was likely a consequence of the 23% decrease ($P < 0.025$) in the

activity of microsomal acyl-CoA:1-acylglycerophosphoethanolamine acyltransferase. Taken together, these results suggest that the remodeling of cardiac phosphatidylethanolamine is regulated by thyroid hormones. A possible role for phosphatidylethanolamine remodeling in the growth and development of heart tissue is postulated.

INTRODUCTION

I. The Biological Membrane

1. Structure of the Biological Membrane

The cell is the basic unit of life. The biological membrane is responsible for organizing the biological processes of the cell. The plasma membrane separates the cell interior from its environment and likewise, the eukaryotic cell interior is further organized into various sub-cellular compartments that are also membrane bound. These include nuclei, mitochondria, the endoplasmic reticulum, Golgi apparatus, lysosomes and peroxisomes. Biological membranes are responsible for numerous cellular functions. These include controlling the secretion and transport of substances, signal transmission, maintaining osmotic balance and restricting the location of cellular events to a particular organelle (Voet and Voet, 1990).

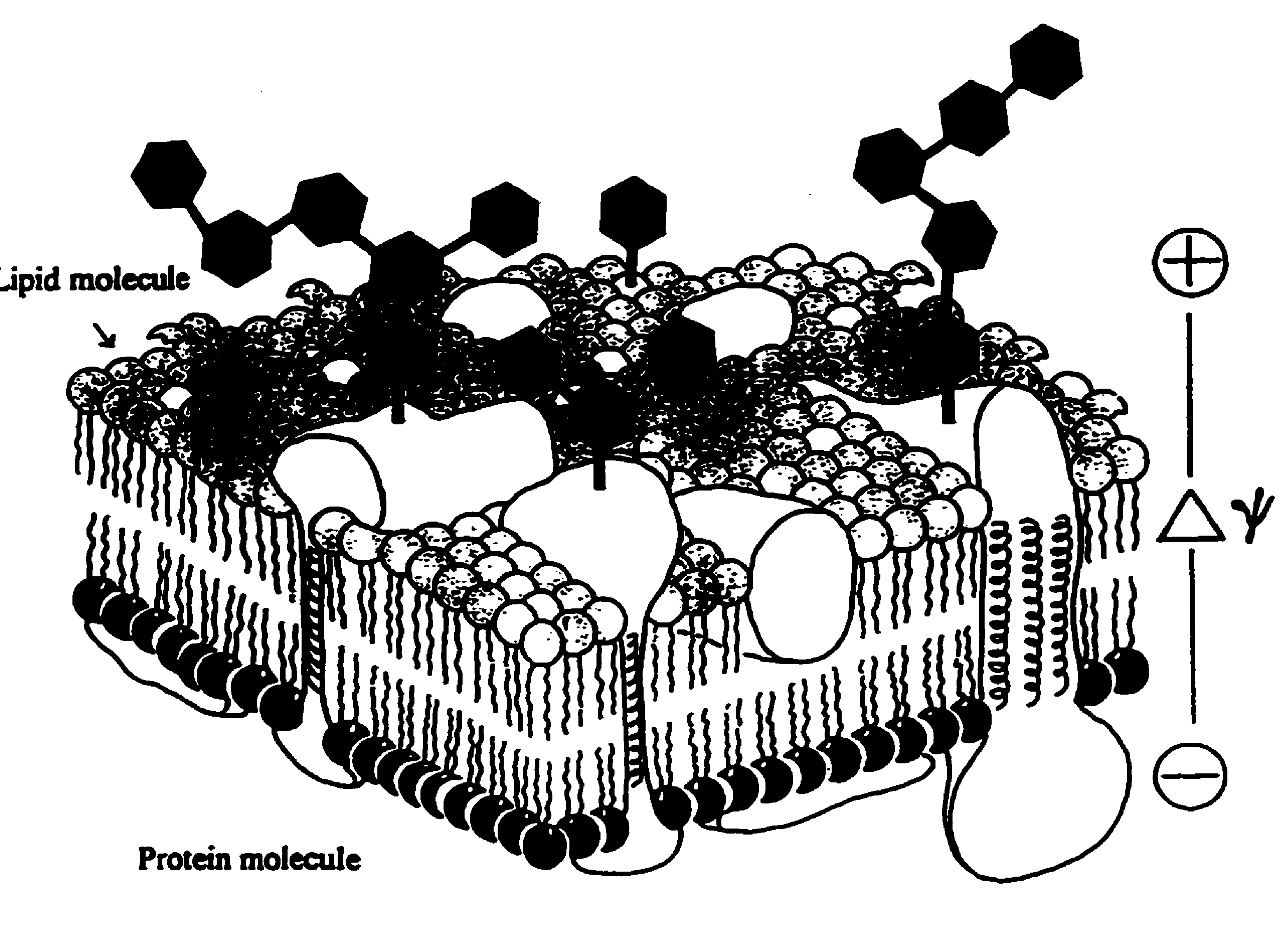
Singer and Nicholson (1972) used artificial lipid bilayers to demonstrate the fluidity of membranes. They developed the fluid mosaic model of membrane structure, whereby free lateral diffusion within the plane of the membrane occurs for both proteins inserted into the membrane as well as the lipids that surround them (Fig. 1).

2. Components of the Biological Membrane

i. Membrane lipids

Membrane lipids are responsible for the establishment of the bilayer structure that has a hydrophobic core with hydrophilic surfaces. This creates a permeability barrier which permits the cell to selectively accumulate organic molecules and ions. Lipids naturally form bilayers due to their amphipathic characteristics. Lipids have both a hydrophilic region (head group) and a hydrophobic region (tail group). The combination of van der Waals forces and hydrophobic interactions stabilizes the biological membrane

Figure 1



Fluid mosaic model of the eukaryotic plasma membrane.

abundant lipid component in membranes. The lipid composition of a given membrane depends upon the type of cell (Diagne *et al.*, 1984). Cholesterol is a major component of the plasma membrane and to a lesser extent in subcellular organelles (Voet and Voet, 1990). Cholesterol functions to determine membrane fluidity since it has a more rigid structure than other lipids. Glycolipids are minor membrane components that are important for cell-cell recognition (Voet and Voet, 1990).

ii. Membrane proteins

The biological membrane is composed of proteins that are associated with a lipid bilayer matrix and these proteins perform most of the membrane functions. However, numerous membrane proteins are found to be regulated by the surrounding lipids (Yeagle, 1989). Proteins that are tightly bound to membranes by hydrophobic forces are defined as integral membrane proteins, whereas proteins associated with a membrane surface through electrostatic or hydrogen bonding interactions are referred to as peripheral membrane proteins. The protein content of membranes varies anywhere from 18% in myelin to 76% in the mitochondrial inner membrane (Guidotti, 1972).

II. Phospholipids

1. Glycerophospholipids

Glycerophospholipids are the major membrane components of membranes. These consist of a glycerol backbone with two fatty acid chains esterified at the *sn-1* and *sn-2* positions (Table I). At the *sn-3* position, an alcohol group is linked to a phosphate that is esterified to a variety of polar head groups (Table II).

2. Plasmalogens

Plasmalogens differ from glycerophospholipids at the *sn-1* position of the glycerol backbone where the fatty acid is attached via an ether linkage (Fig. 2). Plasmalogens are prevalent in tissues such as heart and brain (Diagne *et al.*, 1984).

Table I

Some Common Biological Fatty Acids

Symbol	Common Name	Structure
Saturated fatty acids		
12:0	Lauric acid	$CH_3(CH_2)_{10}COOH$
14:0	Myristic acid	$CH_3(CH_2)_{12}COOH$
16:0	Palmitic acid	$CH_3(CH_2)_{14}COOH$
18:0	Stearic acid	$CH_3(CH_2)_{16}COOH$
20:0	Arachidic acid	$CH_3(CH_2)_{18}COOH$
Unsaturated fatty acids		
16:1	Palmitoleic acid	$CH_3(CH_2)_5CH{=}CH(CH_2)_7COOH$
18:1	Oleic acid	$CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$
18:2	Linoleic acid	$CH_3(CH)_4(CH{=}CHCH_2)_2(CH_2)_6COOH$
18:3	Linolenic acid	$CH_3(CH_2)_4(CH{=}CHCH_2)_3(CH_2)_3COOH$
20:4	Arachidonic acid	$CH_3(CH_2)_4(CH{=}CHCH_2)_4(CH_2)_2COOH$

Table II

Classification of major glycerophospholipids

$$R_2-\overset{\overset{\displaystyle O}{\|}}{C}-O-\underset{\displaystyle CH_2-O-\overset{\overset{\displaystyle O}{\|}}{\underset{\underset{\displaystyle O^-}{|}}{P}}-O-X}{\overset{\displaystyle CH_2-O-\overset{\overset{\displaystyle O}{\|}}{C}-R_1}{CH}}$$

Name of X—OH	Formula of —X	Name of Phospholipid
Water	$-H$	Phosphatidic acid
Ethanolamine	$-CH_2CH_2NH_3^+$	Phosphatidylethanolamine
Choline	$-CH_2CH_2N(CH_3)_3^+$	Phosphatidylcholine (lecithin)
Serine	$-CH_2CH(NH_3^+)COO^-$	Phosphatidylserine
myo-Inositol	(ring structure; labels: H, HO, OH, H, HO, OH, H, H, H, H, OH)	Phosphatidylinositol
Glycerol	$-CH_2CH(OH)CH_2OH$	Phosphatidylglycerol
Phosphatidylglycerol	$-CH_2CH(OH)CH_2-O-\overset{\overset{\displaystyle O}{\|}}{\underset{\underset{\displaystyle O^-}{|}}{P}}-O-CH_2$	Diphosphatidylglycerol (cardiolipin)

Source: Biochemistry (1990), John Wiley & Sons Inc. New York, New York. pp.278-290.

Figure 2

$$
\begin{array}{c}
X \\
| \\
O \\
| \\
O{=}P{-}O^{-} \\
| \\
O \\
| \\
CH_2{-}CH{-}CH_2 \\
| \quad\quad | \quad\quad\quad \\
O \quad\quad O \quad\quad\quad \\
| \quad\quad | \quad\quad\quad \\
CH \quad\quad C{=}O \\
\| \quad\quad | \quad\quad\quad \\
CH \quad\quad R_2 \quad\quad \\
| \quad\quad\quad\quad\quad\quad \\
R_1 \quad\quad\quad\quad\quad\quad
\end{array}
$$

A plasmalogen

Figure 3

Phosphocholine head group:

$$
\begin{array}{c}
CH_3 \\
| \\
CH_3{-}\overset{+}{N}{-}CH_3 \\
| \\
CH_2 \\
| \\
CH_2 \\
| \\
O \\
| \\
O{=}P{-}O^{-} \\
| \\
O
\end{array}
$$

$$
\begin{array}{ccc}
 & H & OH \\
 & | & | \\
CH_2{-} & C{-}{-}{-} & C{-}H \\
 & | & | \\
 & NH & CH \\
 & | & \| \\
 & O{=}C & HC \\
 & | & | \\
 & (CH_2)_{14} & (CH_2)_{12} \\
 & | & | \\
 & CH_3 & CH_3
\end{array}
$$

Palmitate residue: $O{=}C{-}(CH_2)_{14}{-}CH_3$

A sphingomyelin

3. Sphingomyelin

Sphingomyelin has a sphingosine backbone instead of a glycerol backbone (Fig. 3). The sphingosine backbone is formed from the condensation of serine and palmitoyl-CoA (Merrill and Jones, 1990). Fatty acid is attached to the free amino group of the backbone and a phosphocholine head group is attached to the free alcohol group. Sphingomyelin is most prevalent in the myelin sheath that insulates nerve cell axons. Recently, an important role for sphingomyelin hydrolysis in cell signalling has been discovered (Hannun and Bell, 1989).

III. Introduction to Phosphatidylethanolamine

1. Tissue Composition of Ethanolamineglycerophospholipids

In 1930, Rudy and Page (1930) isolated ethanolamine glycerophospholipid from brain tissue. In humans, ethanolamine glycerophospholipids comprised 27.6% of heart, 34.9% of brain, 22% of the lungs, 30.1% of liver, 31.5% of kidney, 31.4% of testis, and 29% of erythrocyte phospholipid phosphorus (Diagne *et al.*, 1984). Ethanolamine glycerophospholipids account for 37.2% of rat and 31.8% of guinea pig phospholipid phosphorus in the heart (Table III). In the heart, the majority of these are diacyl ethanolamine glycerophospholipids (PE), accounting for 14.0, 30.4 and 20.7% of the total lipid phosphorus in the human, rat and guinea pig hearts respectively (Diagne *et al.*, 1984). Alkenylacyl and alkylacyl forms are the next most abundant ethanolamine glycerophospholipids in the heart, with lysophosphatidylethanolamine being the least abundant.

Following only choline glycerophospholipids, ethanolamineglycerophospholipids are quantitatively the most abundant phospholipids in mammalian tissues. Therefore, ethanolamineglycerophospholipids are essential components of cell membranes and have an important structural function.

2. Fatty Acid Molecular Composition of Ethanolamineglycerophospholipids

Fatty acids are esterified to the *sn-1* and *sn-2* positions of the glycerol backbone. The major molecular species of PE in bovine heart (Table IV) reveal that saturated fatty acids are mainly esterified at the *sn-1* position and mainly unsaturated fatty acids to the *sn-2* positon of the glycerol backbone (Scmid and Takahashi, 1968). Stearic acid (18:0) is the most abundant fatty acid attached to the *sn-1* position, whereas arachidonic acid (20:4) is the most abundant fatty acid attached to the *sn-2* position. Alkenyl and alkylacyl ethanolamine glycerophospholipids possess a different fatty acid compositon when

Table III

Phospholipid composition of the heart from human, rat, and guinea pig expressed as a percentage of total lipid phosphorus. Values represent the mean (number of samples).

Phospholipids	Human (2)	Rat (3)	Guinea Pig (3)
Choline glycerophospholipids	41.0 total	41.6 total	43.2 total
diacyl	22.0	39.4	26.5
alkylacyl	2.0	0.6	1.2
alkenylacyl	16.9	1.6	15.5
lysophosphatidylcholine	1.7	0.0	0.3
Ethanolamine glycerophospholipids	27.6 total	37.2 total	31.8 total
diacyl	14.0	30.4	20.7
alkylacyl	1.6	1.2	1.3
alkenylacyl	11.9	5.6	9.8
lysophosphatidylethanolamine	1.6	0.0	0.5
Phosphatidylserine	3.2	1.7	1.7
Phosphatidylinositol	4.3	2.0	2.8
Cardiolipin	14.5	15.0	14.4
Phosphatidic Acid	0.0	0.0	0.0
Sphingomyelin	5.5	3.1	5.1

Source: Biochim. Biophys. Acta (1984) 793, 221-231.

Table IV

Major fatty acid molecular species of phosphatidylcholine and phosphatidylethanolamine in bovine hearts
expressed as a percentage of the total. Values represent the mean.

Fatty Acid	Cholineglycerophospholipid						Ethanolamineglycerophospholipid					
	Diacyl		Alk-1-enyl		Alkylacyl		Diacyl		Alk-1-enyl		Alkylacyl	
	sn-1	*sn-2*	*sn-1*	*sn-2*	*sn-1*	*sn-2*	*sn-1*	*sn-2*	*sn-1*	*sn-2*	*sn-1*	*sn-2*
16:0	62.3	4.8	62.0	2.2	51.8	10.6	3.0	0.5	28.8	1.5	30.5	5.6
18:0	13.9	0.4	11.0	0.7	10.1	1.7	88.1	2.7	42.7	4.4	42.1	5.6
18:1	8.0	28.9	7.0	12.0	18.4	13.1	6.0	3.2	18.1	3.7	18.7	8.8
18:2	3.2	45.5	–	53.0	–	50.7	1.3	13.0	–	34.3	–	17.6
20:4	–	15.9	–	28.2	–	19.5	–	78.4	–	50.1	–	39.2
others	10.8	4.5	18.6	4.4	18.7	4.4	1.6	6.0	6.1	6.0	5.9	23.2

Source: Biochim. Biophys. Acta (1968) 164, 141-147.

compared with diacyl-PE (Table V). The molecular species of PE in the microsomes and mitochondria (Table V) are similar whether rat or human hearts are examined and distinctly different from those of PC (Gloster and Harris, 1970a, b). However, the molecular species of PE in human hearts are quite different from those in rats. For example, arachidonic acid (20:4) is the most abundant fatty acid attached to PE in the human heart, whereas stearic acid (18:0) is the most abundant fatty acid attached to PE in rat heart (Gloster and Harris, 1970a, b). Similarly, the molecular species of PE are different when different tissues of the same animal are compared (Ansell and Spanner, 1982).

3. Biosynthetic Pathways of Phosphatidylethanolamine

i. CDP-ethanolamine pathway

The CDP-ethanolamine pathway (Fig. 4) was first described by Kennedy and Weiss (1956). It has since been shown to be the major route for the biosynthesis of diacyl-PE and ethanolamine plasmalogens in the heart (Arthur and Page, 1991). Initially, ethanolamine is transported into the cell. In the hamster heart, there is a single ethanolamine uptake system whereby uptake of ethanolamine was observed to be linear from 0.1-100 μM exogenous ethanolamine with a K_m of 170 μM for ethanolamine (Zelinski and Choy, 1982). Ethanolamine is rapidly phosphorylated by ATP to form phosphoethanolamine in a reaction catalyzed by ethanolamine kinase. Ethanolamine kinase is a cytosolic enzyme that has been purified from rat kidney (Ishidate *et al.*, 1984) and has a pH optimum of 8-9 and a K_m of 0.6 mM for ethanolamine and 1.5 mM for ATP (Ishidate *et al.*, 1985). Also, an ethanolamine kinase from rat liver has been purified and has a K_m of 1.2 mM for ethanolamine (Porter and Kent, 1990). However, both of these ethanolamine kinase activities contain appreciable choline kinase activity. Recent studies suggest the presence of separate enzymes responsible for the phosphorylation of choline

Table V

Major fatty acid molecular species of phosphatidylethanolamine in human and rat heart microsomes and mitochondria expressed as a percentage of the total. Values represent the mean (number of samples).

Fatty Acid	Phosphatidylethanolamine			
	Microsomes		Mitochondria	
	Human (3)	Rat (6)	Human (3)	Rat (6)
16:0	8.3	11.4	6.1	9.8
18:0	22.3	28.0	26.6	28.6
18:1	9.3	6.4	7.1	6.0
18:2	4.2	6.7	3.4	6.7
20:4	37.1	15.7	37.5	15.2
others	4.1	18.2	0.4	23.7

Source: Cardiovasc. Res. (1969) 3, 45-51; Cardiovasc. Res. (1970) 4, 1-5.

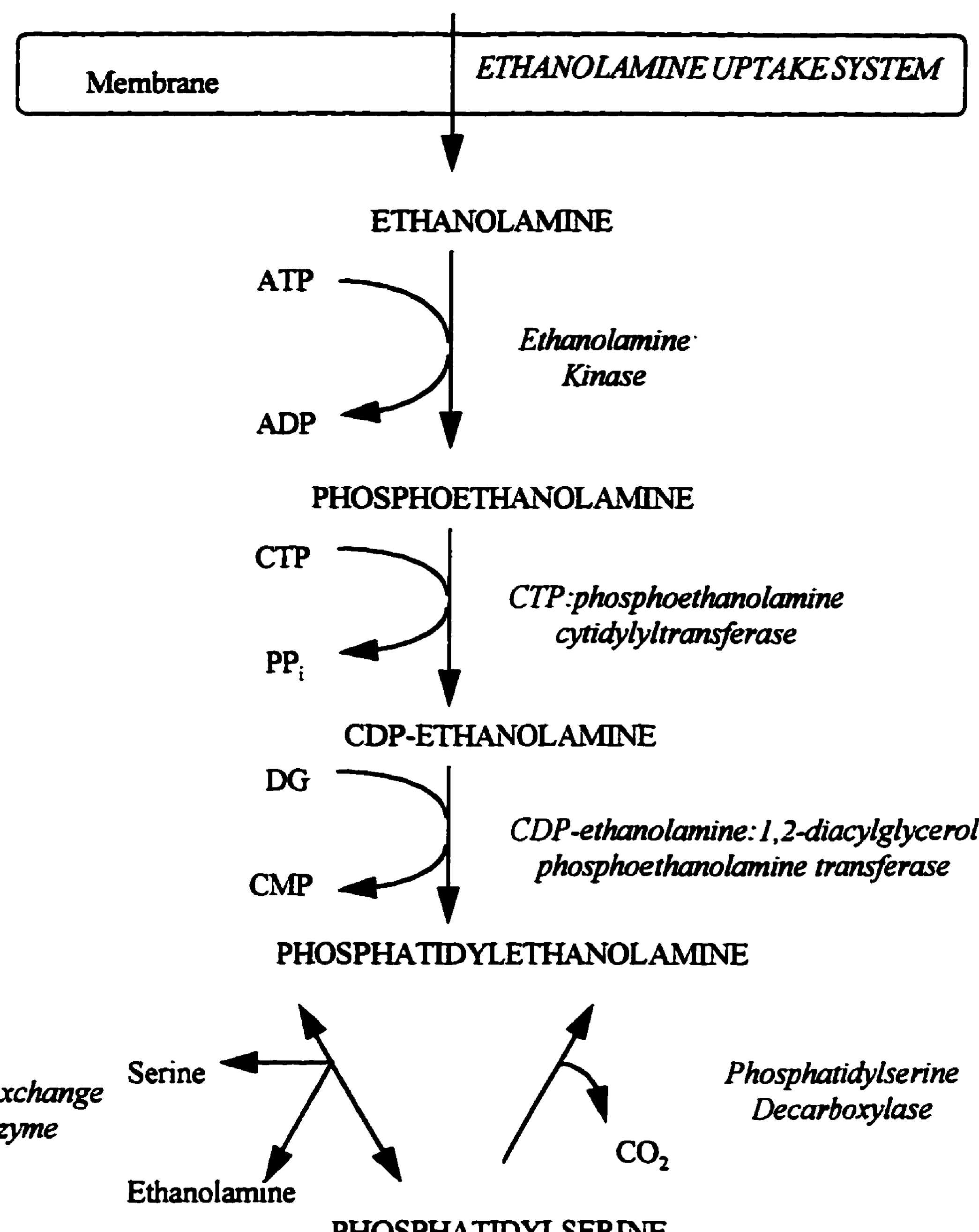
Table VI

Major fatty acid molecular species of phosphatidylcholine in human and rat heart microsomes and mitochondria expressed as a percentage of the total. Values represent the mean (number of samples).

Fatty Acid	Phosphatidylcholine			
	Microsomes		Mitochondria	
	Human (3)	Rat (6)	Human (3)	Rat (6)
16:0	40.9	23.1	39.3	19.8
18:0	9.7	28.2	8.6	26.6
18:1	21.4	10.5	21.2	9.7
18:2	9.3	14.4	10.2	14.0
20:4	9.5	18.1	12.5	21.5
others	5.6	3.8	1.4	5.5

Source: Cardiovasc. Res. (1969) 3, 45-51; Cardiovasc. Res. (1970) 4, 1-5.

Figure 4



Pathways for the *de novo* biosynthesis of phosphatidylethanolamine

and ethanolamine in human liver (Draus *et al.*, 1990) and lens (Ekambaram and Jernigan, 1994).

In the next step, phosphoethanolamine and CTP form CDP-ethanolamine in a reaction catalyzed by CTP:phosphoethanolamine cytidylyltransferase, that has been proposed to be rate limiting (see III. 4. i.). It has been suggested that the formation of CDP-ethanolamine follows a reaction mechanism whereby CTP binds first, followed by phosphoethanolamine (Sundler, 1975). Subsequently, PP_i is released and CDP-ethanolamine is the last product released. This enzyme has been purified from rat liver cytosol as a dimer (Sundler, 1975) and more recently to greater purity (Vermeulen *et al.*, 1993) which showed that it is a single subunit with a molecular mass of 49.6 kDa. CTP:phosphoethanolamine cytidylyltransferase has a pH optimum of 7.8 and a K_m of 65 μM for phosphoethanolamine and 53 μM for CTP (Sundler, 1975).

In the final step of the pathway, CDP-ethanolamine and DG form PE in a reaction catalyzed by CDP-ethanolamine:1,2-DG phosphoethanolaminetransferase. It is an integral membrane protein located on the cytoplasmic side of the endoplasmic reticulum (Vance *et al.*, 1977) although significant activity has been reported in the Golgi apparatus (Vance and Vance, 1988). This enzyme has been partially purified from hamster liver microsomes (19) and was shown to be distinct from cholinephosphotransferase. It has a pH optimum of 8.5 and is activated by Mg^{2+} and Mn^{2+} (O *et al.*, 1989). The apparent K_m for CDP-ethanolamine has been reported to be 22 μM (Kanoh and Ohno, 1976). CDP-ethanolamine:1,2-DG phosphoethanolaminetransferase is active towards both DG and alkenylglycerol (Ansell and Metcalfe, 1971).

ii. Decarboxylation of phosphatidylserine

The formation of PE from the decarboxylation of PS (Fig. 4) was first reported by Bremer et al. (1960) in the rat liver of animals injected with ^{14}C-serine. PS decarboxylase

is located on the inner mitochondria membrane (Voelker, 1989). It has a K_m for PS of 6.5 mM and is dependent upon pyroxidal phosphate (Suda and Matsuda, 1974). In the heart, the decarboxylation of PS has been shown to be used exclusively for the synthesis of diacyl-PE and does not contribute to the synthesis of ethanolamine plasmalogens (Arthur and Page, 1991).

iii. Phosphatidylethanolamine synthesis by Ca^{2+} stimulated base exchange

The Ca^{2+} stimulated exchange of free ethanolamine with the base of PS (Fig. 4) was first described by Borkenhagen et al. (1961) in rat liver homogenates. This activity has been shown to be located on the cytoplasmic side of the endoplasmic reticulum (Bell *et al.*, 1981). The enzyme purified from rat brain microsomes requires Ca^{2+} and has a pH optimum of 7 (Suzuki and Kanfer, 1985). It has K_m values of 20 µM for ethanolamine and 110 µM for serine.

Base-exchange has been deemed not to play an important role *in vivo* in PE biosynthesis (Yabusaki and Wells, 1973), however base exchange may account for up to 10% of PE synthesis in the heart (Zelinski and Choy, 1982). In the heart, over 90% of *de novo* synthesized PE is synthesized via the CDP-ethanolamine pathway (Zelinski and Choy, 1982; Arthur and Page, 1991). Much of the remaining PE is synthesized via the decarboxylation of PS. A Chinese Hamster ovary mutant (Miller and Kent, 1986) and BHK-21 cell line (Voelker, 1984) that were defective in the CDP-ethanolamine pathway were able to derive PE solely from PS decarboxylation. The relative contributions of these different pathways to the total PE synthesized varies among different tissues and cell types. However, the relative contributions of each of these pathways to PE biosynthesis may also change under different physiological conditions.

4. Regulation of Phosphatidylethanolamine Biosynthesis

i. Regulation by ethanolamine and serine

The rate limiting step of PE biosynthesis was shown to be the formation of CDP-ethanolamine by CTP:phosphoethanolamine cytidylyltransferase. This was determined in experiments where hepatocytes that were exposed to increasing ethanolamine concentrations incorporated more labeled glycerol into PE, but the CDP-ethanolamine pool size was unchanged (Sundler and Akesson, 1975). Studies using perfused hamster hearts provided further evidence for the rate limiting role of CTP:phosphoethanolamine cytidylyltransferase (Zelinski and Choy, 1982).

However, increasing the exogenous ethanolamine concentration in the perfusate was able to shift the rate limiting step of the CDP-ethanolamine pathway from the reaction catalyzed by CTP:phosphoethanolamine cytidylyltransferase to the reaction catalyzed by ethanolamine kinase in the perfused hamster heart. At concentrations of 0.04-0.1 μM ethanolamine most of the radioactivity was associated with phosphoethanolamine, but at concentrations of 0.4-1000 μM ethanolamine, most of the radioactivity was associated with ethanolamine without changing the intracellular ethanolamine pool (McMaster and Choy, 1992a). This result suggested that newly imported ethanolamine does not mix with the endogenous ethanolamine pool and the exogenous ethanolamine is used preferentially for PE biosynthesis. Furthermore, the use of the ethanolamine analogue, monomethylethanolamine, inhibited ethanolamine uptake as well as the ethanolamine kinase reaction in the perfused guinea pig heart (McMaster *et al.*, 1992). Ethanolamine uptake and ethanolamine kinase must be tightly coupled in the heart. Therefore, the heart uses ethanolamine kinase during high ethanolamine uptake as an additional mechanism for maintaining the appropriate rate of PE biosynthesis. This mechanism appears to be at work in rat liver as well since an increase in the ethanolamine concentration in the serum

following partial hepatectomy caused an increase in PE biosynthesis and an accumulation of ethanolamine (Houweling *et al.*, 1992).

The contributions of the PS decarboxylase and CDP-ethanolamine pathways to PE biosynthesis has been shown to be modulated by the supply of ethanolamine and serine. In human retinoblastoma cells (Yorek *et al.*, 1985), rat hepatocytes (Tijburg *et al.*, 1989) and bovine aortic cells (Lipton *et al.*, 1990), the presence of ethanolamine in the medium reduced labeling of PE from the decarboxylation of PS. In rat hepatocytes, addition of serine to the medium did not affect the incorporation of ^{14}C-ethanolamine into PE (Tijburg *et al.*, 1989a). However, in hamster hearts perfused with 50 μM ethanolamine, the presence of 0.05-10 mM serine was able to selectively inhibit the incorporation of ^{3}H-ethanolamine into PE (McMaster and Choy, 1992b). Serine was able to inhibit ethanolamine uptake and the activity of ethanolamine kinase without affecting the decarboxylation of PS. The modulation of PE biosynthesis by ethanolamine and serine may be physiologically important since the circulating levels of ethanolamine (0.01-0.9 mM) and serine (0.03-0.4 mM) fluctuate (Milakofsky *et al.*, 1985).

ii. Regulation by diacylglycerol

DG is a precursor of PE that may be produced by the action of phosphatidic acid phosphatase upon phosphatidic acid and the hydrolysis of membrane phospholipids by phospholipase C. DG is located at the branch in the pathway between acidic phospholipid biosynthesis and neutral lipid biosynthesis. It has been postulated that more than one pool of DG exists within the cell and *de novo* DG pools are distinct from existing DG pools (Rustow and Kunze, 1985, 1987). Therefore, the availability of DG may be a determinant of the rate of PE biosynthesis. Treatment of hepatocytes with the phorbol ester, phorbol myristate acetate increased ^{14}C-ethanolamine incorporation into PE as well as the activites of CTP:phosphoethanolamine cytidylyltransferase and CDP-ethanolamine:1,2-DG

phosphoethanolaminetransferase (Tijburg *et al.*, 1987a). It was suggested that a 50% increase compared with controls in the hepatocyte DG pool size in the presence of phorbol ester was responsible. When hepatocytes were treated with Okadaic acid, a protein phosphatase 1 and 2A inhibitor, PE biosynthesis was inhibited, without changing the activity of CDP-ethanolamine:1,2-DG ethanolaminephosphotransferase (Tijburg *et al.*, 1992). Since the DG level was reduced in Okadaic acid treated hepatocytes by 30% of controls, the diminished DG supply was suggested to be responsible for the inhibition of PE biosynthesis.

iii. Regulation by hormones

By binding specific receptors on the cell surface, hormones are able to influence intracellular events. The hormone, norepinephrine, was able to inhibit ^{14}C-ethanolamine incorporation into PE in rat hepatocytes (Haagsman *et al.*, 1984). Since phenoxybenzamine was able to abolish the inhibition caused by norepinephrine, control of PE biosynthesis by norepinephrine is likely mediated through an α-adrenergic receptor.

Vasopressin is a Ca^{2+} dependent peptide hormone secreted by the posterior lobe of the pituitary that mediates its effects through the vasopressin receptor. In rat hepatocytes, vasopressin stimulated ^{14}C-ethanolamine incorporation into PE compared to controls (Tijburg *et al.*, 1987b). Since vasopressin stimulates PI hydrolysis and resulting in the formation of DG, the increased PE synthesis may actually be due to the increased DG accumulation in vasopressin treated cells (Bocckino *et al.*, 1985).

Glucagon is a polypeptide involved in the mobilization of hepatic glucose. Exposure of hepatocytes to glucagon decreased ^{14}C-ethanolamine incorporation into PE compared with controls (Tijburg *et al.*, 1989b). However, the activity of the enzymes of the CDP-ethanolamine pathway were unaffected. The inhibitory effect of glucagon on PE

synthesis was likely due to the observed diminished pool size of DG in the glucagon treated hepatocytes compared with untreated cells.

IV. Remodelling of Phospholipids

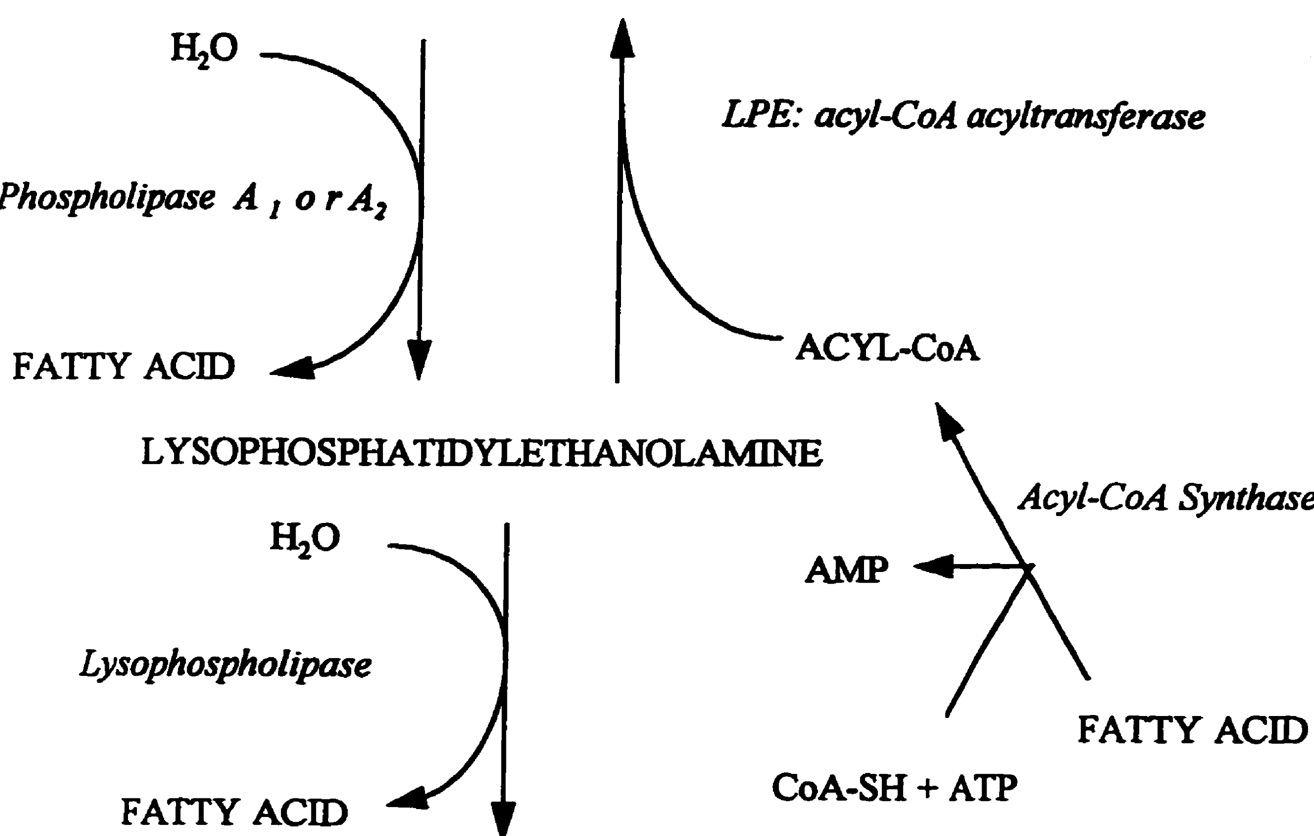
The biological membrane is a dynamic structure. Phospholipids are continuously degraded at the same time as newly synthesized phospholipids are added. In 1960, Lands (1960) described the deacylation-reacylation cycle for the remodelling of PC. It was proposed that enzymes existed that were capable of hydrolyzing and reacylating fatty acids on the phospholipid glycerol backbone (Fig. 5). Phospholipids are continuously remodelled within the membrane in order to adjust and maintain their fatty acid molecular composition. Phospholipid remodelling is very important since tailoring newly synthesized phospholipids to the requirement of the particular tissue can affect the fluidity and permeability of the membrane, as well as regulate membrane bound enzyme activities. In addition, phospholipid remodelling may provide a mechanism for repairing lipids damaged by oxidative destruction. Remodelling of phospholipids must be carefully balanced between the deacylating and reacylating activities otherwise the normal functioning of the membrane will be disturbed.

1. Enzymes Involved in Phospholipid Remodelling

i. Catabolic Enzymes

Phospholipase A_1 and A_2 have an important role in the catabolism of membrane phospholipids and are widely distributed in mammalian tissues (van den Bosch, 1982). Phospholipase A_1 catalyzes the hydrolysis of the acyl group from the *sn-1* position of the phospholipid glycerol backbone to release a fatty acid and the corresponding 2-acyl-lysophospholipid (Fig. 6). Phospholipase A_2 catalyzes a similar reaction at the *sn-2* position of the phospholipid glycerol backbone (Fig. 6). Intracellular phospholipase A_2 has been purified from human U937 cells, its protein sequence determined and the cDNA has been cloned (Kramer *et al.*, 1986; Clark *et al.*, 1990; 1991). It is a 85 kDa protein that is activated at μM concentrations of Ca^{2+} and has a preference for arachidonic acid

Figure 5



Remodelling pathway of phosphatidylethanolamine

Figure 6

Phospholipase A_1

Phospholipase A_2

H_2O

$R_1-C(=O)-O^-$

$R_2-C(=O)-O^-$

2-acyl lysophospholipid

1-acyl lysophospholipid

Lysophospholipase A_2

Lysophospholipase A_1

Glycerophospholipid

Catabolic enzymes

containing phospholipids. A Ca^{2+} independent phospholipase A_2 has also been isolated from canine myocardium (Wolf and Gross, 1984; Hazen *et al.*, 1990). It is a 40 kDa protein that has a preferenece for arachidonic acid containing phospholipids. Phospholipase A_2 is particularly important due to its role in inflammation, cell proliferation and signal transduction (Mukherjee *et al.*, 1994). Removal of fatty acid from the *sn-2* position of newly synthesized phospholipids is necessary for the subsequent introduction of polyunsaturated fatty acids (Irvine, 1982). Specificity studies on long-chain acyl groups at the *sn-2* position reveals that linoleic acid (18:2) is released faster than linolenic (18:3) or arachidonic (20:4) acid (Woelk *et al.*, 1974).

Lysophospholipids account for only 1-2% of the total phospholipid mass in mammalian tissues (Zubay, 1988). However, due to their cytolytic (Weltzien, 1979) and arrhythmia generating properties (Kinnaird *et al.*, 1988), the metabolism of lysophospholipids must be tightly regulated in all tissues and especially the heart. The lysophospholipid product of phospholipase A activity may be resynthesized (see IV. 1. ii.) or further catabolized through the action of a lysophospholipase. In fact, the LPC level in rat and guinea pig heart microsomes appears to be regulated by the activity of the microsomal lysophospholipase (Giffin *et al.*, 1986). Lysophospholipase A_1 hydrolyzes acyl groups from a 1-acyl-, and lysophospholipase A_2 from a 2-acyl-lysophospholipid to release fatty acid and a glycerol-3-phosphate compound with its corresponding base attached (Fig. 6). Lysophospholipase activity that attacked both LPC and LPE was first demonstrated in rat liver by Dawson (1956). Lysophospholipases have been shown to exist in both the microsomal and cytosolic fractions of heart tissue (Gross and Sobel, 1981). Lysophospholipase was purified from rabbit myocardial cytosol and was shown to have a molecular weight of 23 kDa (Gross and Sobel, 1982). This enzyme had a K_m of 10 μM for 1-acyl-LPC and a pH optimum of 7.5. It did not show any preference for LPC

substrates with different acyl groups. However, a lysophospholipase A_2 activity has been shown to exist in guinea pig heart microsomes that has a preference for 2-linoleoyl-LPC and 2-arachidonyl-LPC over other LPC substrates (Arthur, 1989a). Therefore a combination of phospholipase A_1 followed by lysophospholipase A_2 activities may be involved in the release of linoleic (18:2) and arachidonic (20:4) acids from phospholipids. Recently, a 24 kDa lysophospholipase was purified from rat liver and its cDNA cloned (Sugimoto *et al.*, 1996). The transcript was found to exist in heart tissue, among others. The enzyme was active over a wide pH range of 5.5-9.0 and the K_m for 1-palmitoyl-LPC was determined to be 170 μM. It utilized other lysophospholipids as well and exhibited significant activity towards LPE substrates.

ii. Resynthetic enzymes

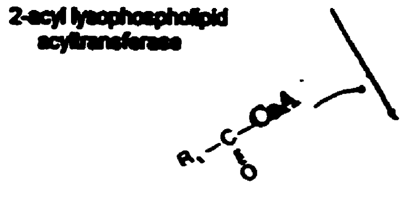
Enzymes involved in the resynthesis of phospholipids are necessary for the removal of lysophospholipids as well as determining the desired fatty acid composition of the particular phospholipid. Prior to their addition to the phospolipid glycerol backbone, fatty acids must be activated. Acyl-CoA synthetase catalyzes a reaction whereby ATP is required to attach CoA to the fatty acid to produce a fatty acyl-CoA, PP_i and AMP (Fig. 5) (Zubay, 1988). These enzymes do not have a preference for fatty acids (Marcel and Suzue, 1972).

The transfer of acyl groups from acyl-CoA to 1-acyl-lysophospholipid is catalyzed by acyl-CoA:1-acyl-lysophospholipid acyltransferase to produce the corresponding diacylphospholipid (Fig. 7). The transfer of acyl groups from acyl-CoA to 2-acyl-lysophospholipid is catalyzed by acyl-CoA:2-acyl-lysophospholipid acyltransferase (Fig. 7). The existence of two separate enzymes in liver and heart for the reacylation of LPC has been established (Lands and Merkl, 1962; Arthur, 1989b). Palmitoyl-CoA and stearoyl-CoA are preferentially esterified to 2-acyl-LPC whereas linoleoyl-CoA and oleoyl-CoA

Figure 7

2-acyl lysophospholipid

1-acyl lysophospholipid



1-acyl lysophospholipid
acyltransferase

Diacyl Phospholipid

Resynthetic enzymes

are preferentially esterified to 1-acyl-LPC in the liver (Lands and Merkl, 1962). Rat liver microsomes also possess two separate activities for the reacylation of 1-acyl- and 2-acyl-LPE. It has been shown that linoleoyl-CoA is the preferred substrate for the resynthesis of 1-acyl-LPE whereas stearoyl-CoA is the preferred substrate for the resynthesis of 2-acyl-LPE (Merkl and Lands, 1962). However, most reacylation studies have focused on the acylation of 1-acyl-lysophospholipids. The existence of two separate acyltransferase activites is likely important for maintaing the asymmetric distribution of phospholipid fatty acid molecular species.

The purification of acyltransferase enzyme from various tissues has been reported. An acyltransferase from bovine brain microsomes that had a strong preference for LPC as its substrate has been purified (Deka *et al.*, 1986). It was reported to have a molecular weight of 43 kDa, preferred arachidonyl-CoA as a substrate and exhibited no activity towards long chain saturated fatty acids. An LPC AT from rabbit lung was shown to have an iso-ping-pong kinetic mechanism (Arche *et al.*, 1987). It had a K_m of 8.5 µM for palmitoyl-CoA and 61 µM for LPC. A 63 kDa LPC AT was purified from bovine heart microsomes. 1-Palmitoyl-LPC and linoleoyl-, arachidonyl- and oleoyl-CoAs were its preferred substrates, but K_m values were not determined (Sanjanwala *et al.*, 1988). However, in the case of all these purified enzymes, no follow-up studies elucidating the protein sequence or regulatory mechanisms exist.

2. Remodelling of Phosphatidylethanolamine

Structural analysis of PE indicates that saturated fatty acids are esterified at the *sn-1* and polyunsaturated fatty acids at the *sn-2* position (Holub and Kuksis, 1978). The observed fatty acid distribution does not result from acyl selectivity of DGs by rat liver microsomal CDP-ethanolamine:1,2-DG ethanolaminephosphotransferase (de Kruiff *et al.*, 1970). Rat liver slices and *in vivo* studies of rat liver have shown that *de novo* synthesis

produces mainly monoenoic and dienoic PEs whereas polyunsaturated PEs were produced by the remodelling reactions (van Golde *et al.*, 1969; Trewhella and Collins, 1972). It has been shown that *in vivo*, almost all of the linoleic (18:2) and arachidonic (20:4) acid entered hepatic PE by the acylation of LPE (Akesson, 1970). These results indicate that the asymmetry in the PE fatty acid composition is established by phospholipid remodelling reactions (Holub and Kuksis, 1978). The remodelling of PE was first reported by Merkl and Lands (1962) to occur in rat liver microsomes. Somewhat inconsistent with this model was the finding that there was no increase in the total radioactivity associated with the six major molecular species of PE over a 24 hour period in rat hepatocytes (Samborski *et al.*, 1990). This result suggested that no significant remodelling of PE derived from the CDP-ethanolamine pathway occurs in rat hepatocytes. However two later reports demonstrated substantial turnover of hepatic PE and PE derived from PS decarboxylation (Schmid *et al.*, 1991; Samborski and Vance, 1992). Using rat hepatocytes incubated with either ^{14}C-glycerol or $H_2{}^{18}O$ it has been shown that to synthesize the major fatty acid species of PE, (18:0-20:4), it is necessary for remodelling reactions to occur at both the *sn-1* and *sn-2* positions (Schmid *et al.*, 1995). The main PE fatty acid combination derived from the *de novo* pathway (16:0-22:6) undergoes remodelling to produce the primary remodelling products (16:0-20:4; 18:0-22:6) and then further remodelling of these results in the final the stable secondary remodelling product (18:0-20:4).

PE made by the CDP-ethanolamine pathway is degraded rapidly in rat hepatocytes (Samborski *et al.*, 1990). This may occur since microsomal LPE must compete with LPC for acyl-CoA. LPC is reacylated at a higher rate than LPE, leaving LPE exposed to hydrolysis by microsomal lysophospholipase in rat liver (Pind *et al.*, 1984).

In the heart, LPE AT activity is mainly located in the microsomal fraction (Arthur *et al.*, 1987a), however LPE AT activity is also present in cardiac mitochondria (Arthur *et*

al., 1987b). In guinea pig heart, both mitochondrial and microsomal LPE AT exhibited a broad pH optimum of 6-9 (Arthur *et al.*, 1987a,b). Microsomes have a preference for long-chain acyl-CoAs in the order of linoleoyl > arachidonyl >> palmitoyl > oleoyl > stearoyl-CoA (Arthur *et al.*, 1987a). Conversely, mitochondrial LPE AT has preference for long-chain acyl-CoAs in the order of arachidonyl > linoleoyl > palmitoyl > oleoyl = stearoyl-CoA (Arthur *et al.*, 1987b). Ions had different effects on LPE AT in different sub-cellular fractions since microsomal LPE AT was inhibited by 5 mM Ca^{2+} whereas mitochondrial LPE AT was activated by 5 mM Ca^{2+}. The microsomal enzyme was inhibited by the sulfhydryl binding reagents iodoacetate and N-ethylmaleimide.

3. Regulation of Phospholipid Remodelling

Tight regulation of phospholipid remodelling is necessary to prevent accumulation of lysophospholipids or phospholipids that contain an inappropriate fatty acid composition. The elucidation of the regulatory mechanisms underlying phospholipid remodelling would clarify the probelm as to how the biological membrane preserves a relatively constant lipid composition and physical properties.

i. Effect of membrane environment on the enzymes involved in phospholipid remodelling

The lipid environment surrounding membrane bound enzymes can influence their activities (Kimelberg, 1957; Coleman, 1972). Both phospholipase A_2 and the acyltransferases use the membrane as their residence as well as their substrate. The influence of the lipid environment on these enzymes could play an important role in the maintenance of optimal membrane fluidity. For example, liver phospholipid fatty acid composition was changed by feeding rats diets that contained different saturated lipids. This altered liver plasma membrane fluidity and consequently changed the activity of phospholipase A_2 (Momchilova *et al.*, 1984).

Phospholipase A_2 activity in both liver plasma and microsomal membranes depends upon the fatty acid composition of the surrounding phospholipids. Modification of membrane fluidity by butanol treatment or different degrees of delipidation by phospholipases was used to examine the effect of changing membrane fluidity on phospholipase A_2 activity. Phospholipase A_2 activity towards exogenous substrates in the liver increased when the rigidity of the membrane was increased and decreased when the fluidity of the membrane was increased (Momchilova *et al.*, 1985; 1986). When the liver phospholipid composition was modified using lipid transfer proteins to change the content of dipalmitoyl-PC (increases membrane rigidity) and dioleoyl-PC (increases membrane fluidity), phospholipase A_2 activity towards endogenous substrates was affected in the same manner by the membrane environment (Petkova *et al.*, 1987). Acyltransferases were affected by membrane fluidity in a different manner. Use of lipid transfer proteins or partial delipidation with phospholipases to modify membrane physical properties markedly affected the activity of rat liver LPC AT. Increased membrane fluidity increased LPC AT activity whereas increased rigidity caused a decrease in LPC AT actvity in both plasma (Momchilova *et al.*, 1991) and microsomal membranes (Koshlukova *et al.*, 1992). Similarly, LPE AT activity was also increased when membrane fluidity is increased and increased membrane rigidity decreased LPE AT activity in rat liver plasma membranes (Momchilova-Pankova *et al.*, 1990). Therefore a similar mechanism must exist for acyltransferases and phospholipase A_2 to respond to changes in the membrane fluidity. It is necessary for both of these enzymes to respond to the physical state of their surrounding membrane environment, since their function involves the remodelling of membrane phospholipids for the maintenance of optimal structure and function of the biological membrane.

ii. Regulation of phospholipase A_2

The regulation of intracellular phospholipase A_2 is rather complex. Several mechanisms appear to exist, including regulation by enzyme phosphorylation, modulation of the free Ca^{2+} concentration, as well as by its products and substrates.

There is growing evidence for regulation of intracellular phospholipase A_2 by protein phosphorylation. Stimulation of receptors leads to activation of MAP kinase, phosphorylating phospholipase A_2, thereby activating it. Phosphorylation of serine residues on phospholipase A_2 was increased both consitutively and in response to cell stimulation in different cell systems (Wijkander and Sundler, 1991; Lin *et al.*, 1993; Nemenoff *et al.*, 1993). The increased phosphorylation coincided with an increase in phospholipase A_2 activity. Experiments using protein kinase C inhibitors and activators suggest that MAP kinase is activated by protein kinase C to phosphorylates phospholipase A_2 (Posacla and Cooper, 1992; Seger and Krebs, 1995). However, the protein kinase C-α isoform can cause arachidonate release, independent of MAP kinase activation (Xing *et al.*, 1997). Phospholipase A_2 has also been shown to be phosphorylated on tyrosine residues in HEL-30 keratinocytes by transforming growth factor-α (Kast *et al.*, 1993). Recent reports have cast doubt on the importance of phospholipase A_2 phosphorylation. The inhibition of p38 MAP kinase inhibits phospholipase A_2 phosphorylation without inhibiting phospholipase A_2 catalyzed arachidonic acid (20:4) release (Ambs *et al.*, 1995; Kramer *et al.*, 1996).

The normally cytosolic phospholipase A_2 is translocated to the cell membrane upon cellular activation in the presence of μM concentrations of Ca^{2+} (Clark *et al.*, 1991; Channon and Leslie, 1989). Phospholipase A_2 contains a Ca^{2+} dependent membrane interfacial binding site, separate from the active site that is responsible for the translocation and association of the enzyme with phospholipids in the cell membrane. Rat liver phospholipase A_2 activity towards mitochondrial PE showed 1 activity plateau upon

the variation of the Ca^{2+} concentration suggesting that the enzyme has one Ca^{2+} binding site (Lenting *et al.*, 1987). The rat liver phospholipase A_2 has a K_m of 0.05 mM for Ca^{2+}, which is within the range for the intramitochondrial Ca^{2+} concentration. This value suggests that phospholipase A_2 could be regulated by the free Ca^{2+} concentration alone. However, phospholipase A_2 can be activated by other divalent metal ions as well, suggesting that the physiological role of the metal ion is merely to promote association of the cytosolic enzyme with the phospholipid substrate rather than serving in catalysis (Dennis, 1994).

Phospholipase A_2 may also be regulated by its substrates and products. Addition of 8 nM CL or PG was able to stimulate rat liver mitochondrial phospholipase A_2 hydrolysis of PE and PC (Reers and Pfeiffer, 1987). This may be due to these negatively charged phospholipids having a high affinity for Ca^{2+} which creates a higher local Ca^{2+} concentration in the mitochondrial phospholipase A_2 surroundings. Conversely, MLCL and DLCL have been shown to be very specific and potent inhibitors of phospholipase A_2 (Lenting *et al.*, 1987). The low inhibitor concentration is evidence for MLCL directly interacting with phospholipase A_2 to block its activity. Linoleic acid was able to inhibit mitochondrial phospholipase A_2 (De Winter *et al.*, 1982). In the mitochondria, linoleic acid would be a major product of phospholipase A_2 towards CL. Since endogenous CL has been shown to be resistant towards phospholipase A_2 hydrolysis (De Winter *et al.*, 1986), inhibition by linoleic acid could have an important role in blocking phospholipase A_2 activity against endogenous CL. In various cell lines phospholipase A_2 has been shown to be inhibited by endogenous unsaturated fatty acids (De Winter ., 1982; Wood *et al.*, 1985; Ballou and Cheung, 1983; 1985). In addition, glycerol has also been shown to enhance the activity of phospholipase A_2 (Reynolds *et al.*, 1992; Burke *et al.*, 1995). The relevance of this is unknown, however glycerol may act as a regulatory factor that

enhances remodelling reactions or its presence could act to change the properties of the membrane bilayer. Therefore, the substrates and products of phospholipase A_2 may act in regulating its activity, thereby controlling the rate phospholipid remodelling in various membranes.

iii. Regulation of acyltransferase

Much less is known about how the phospholipid acyltransferases are regulated, however it is likely that their regulation must be closely tied to the regulation of phospholipase A activity in order to maintain the balance of deacylation-reacylation. However, high concentrations of subtrates such as LPC and palmitoyl-CoA are inhibitory towards LPC AT in lung microsomes (Hasegawa-Sasaki and Ohno, 1975). Divalent cations that activate phospholipase A_2, such as Ca^{2+}, have been shown to inhibit LPC AT in rat submandibular gland and guinea pig hearts (Arthur and Choy, 1986; Yashiro *et al.*, 1989). The hypolipidemic drugs, WY-14643 and clofibric acid, inhibit mitochondrial LPC AT (Riley and Pfeiffer, 1986). Clofibric acid administration affects increased microsomal LPC AT activity in liver, kidney and intestinal mucosa, but did not affect activity in the heart (Kawashima *et al.*, 1986). Chronic adminstration of the β-adrenergic agonist, isoproterenol, inhibited rat microsomal LPC AT (Yashiro *et al.*, 1989). When cells are activated, there is an immediate increased turnover of phospholipid unsaturated fatty acids. The hormone, bradykinin can regulate this effect through stimulation of phospholipase A_2, followed by an increase in acyltranferase activity (Sterin-Speziale *et al.*, 1989). Similarly, the cytokine interleukin-1α has the ability to stimulate phospholipase A_2, while at the same time stimulating acyltransferase activity (Nakazato and Sedor, 1992). In various rat tissues acyltransferase activity has been shown to be inhibited by detergents and inhibitors of cyclic nucleotide phosphodiesterase (Shier, 1977). Since phosphatase treatment of human neutrophils decreased acyltransferase activity, it has been

postulated that acyltransferase may be regulated by phosphorylation-dephosphorylation (Reinhold *et al.*, 1989).

Much remains to be understood about the coordination of *de novo* phospholipid biosynthesis and phospholipid remodelling. Recent experiments using hepatocytes and macrophages incubated with $H_2{}^{18}O$ to measure uptake of ^{18}O into phospholipid acyl groups has shown that *de novo* biosynthesis and phospholipid remodelling occur at similar rates (Schmid *et al.*, 1995; Kuwae *et al.*, 1997). These processes must be tightly regulated so that they remain balanced.

V. Thyroid Hormones

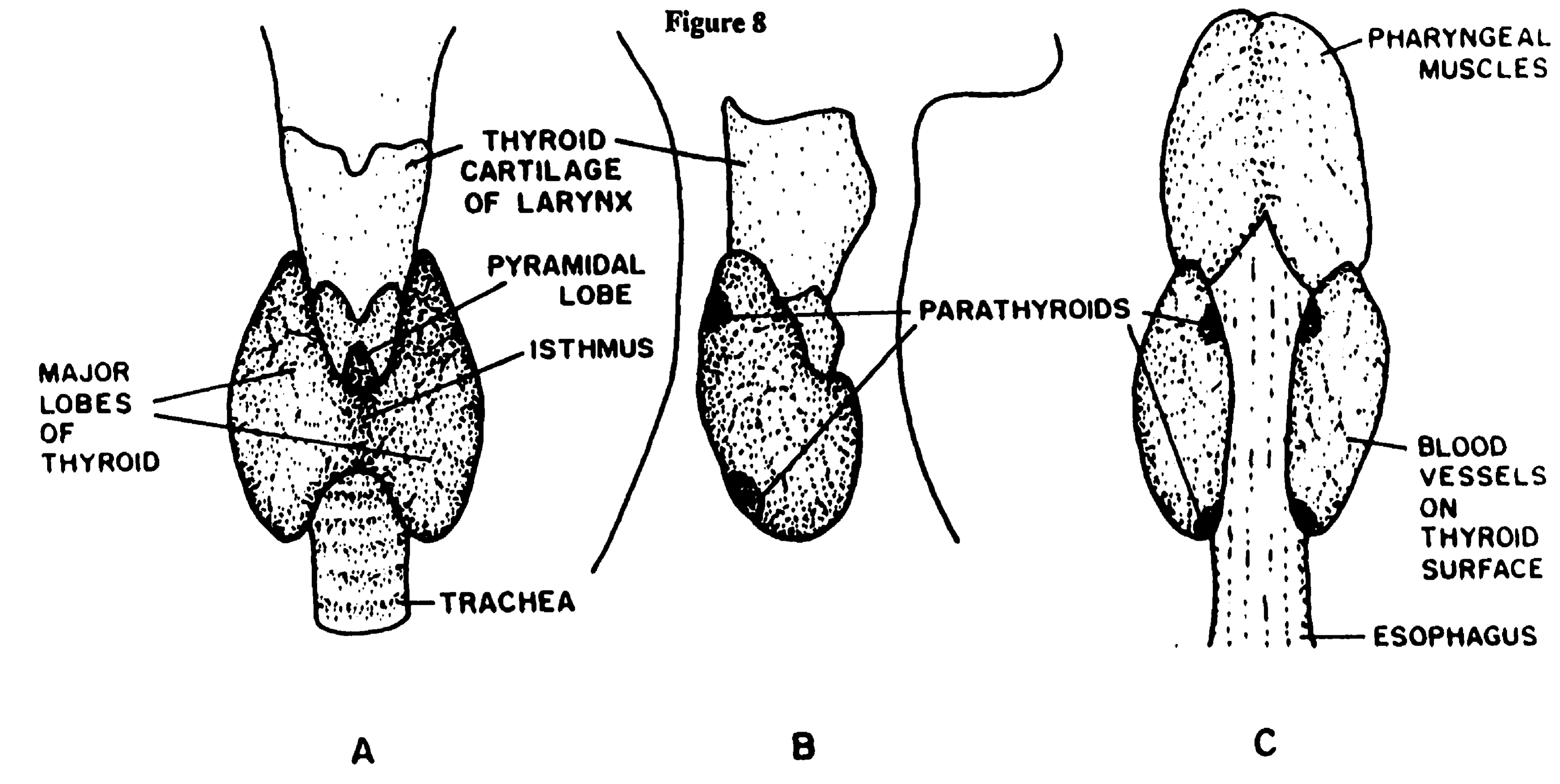
1. Thyroid hormone structure and synthesis.

In recent years, a great deal of understanding about thyroid hormones and their physiological activities has been gained. Thyroid hormones are intercellular messengers that coordinate cellular developmental and metabolic events in many tissues (Ichikawa and Hashizume, 1995; McNabb, 1992). For example, thyroid hormones control metabolism in homeotherms by reducing the basal metabolic rate in the hypothyroid state and elevating the basal metabolic rate in the hyperthyroid state (McNabb, 1992).

Thyroid hormones are produced in the thyroid gland that is located in the neck on the ventral and lateral surface of the trachea (Fig. 8). The main thyroid hormones are T_3 and T_4 (Fig. 9). T_4 was the first to be isolated from thyroid tissues in 1915 (Kendall, 1915) and T_3 was isolated in 1952 (Gross and Pitt-Rivers, 1952). Quantitatively, T_4 is the most abundant form in the serum at a concentration of 100 nmole/L whereas T_4 is present at a concentration of 1.7 nmole/L (McNabb, 1992). However, most thyroid hormone action is due to T_3, which has a physiological potency that is seven times that of T_4 (McNabb, 1992). Most of T_3 is produced in extrathyroidal tissues and 85% arises from the deiodination of T_4 catalyzed by the enzyme 5' deiodinase which is located within the microsomal fraction (Ichikawa and Hashizume, 1995).

Thyroid hormones are initially synthesized on the ribosomes of the rough endoplasmic reticulum within thyroid gland follicle cells as the prohormone, thyroglobulin (McNabb, 1992). This protein is glycosylated within the endoplasmic reticulum and after undergoing processing within the Golgi apparatus, it is transported within exocytotic vesicles to the follicle cell lumen. Tyrosine residues on thyroglobulin are iodinated within the lumen adjacent to the apical membrane (Ekholm, 1981). Peroxidase catalyzes the coupling of two diiodotyrosine molecules through the elimination of an alanine side chain

Figure 8



Anatomy of the human thyroid gland.

A) Ventral view, B) Lateral view, C) Dorsal view

Figure 9

TRIODOTHYRONINE (T3)

3,5,3'-TRIIODOTHYRONINE

THYROXINE (T4)

3,5,3',5'-TETRAIIODOTHYRONINE

Thyroid Hormone Structures

to form a tetraiododthyronine or T_4 (McNabb, 1992). Thyroid hormones are then stored as thyroglobulin within the extracellular lumen of the thyroid follicle. Storage provides a long-term pool of thyroid hormones that can be removed from the extracellular lumen upon stimulation of follicle cells by thyroid stimulating hormone. Thyroglobulins are degraded within phagolysosomes to produce free thyroid hormones which then diffuse into the capillary circulation (Dunn and Dunn, 1988).

Only 0.03% of thyroid hormone in the serum exists in a free form (Ichikawa and Hashizume, 1995). Most thyroid hormones are bound to one of three proteins: thyroxine-binding globulin (68%), thyroxine-binding pre-albumin (11%) and albumin (20%) (McNabb, 1992). These proteins are important for maintaining a pool of thyroid hormone within the bloodstream that is not removed by the kidney and regulating the delivery of thyroid hormones to the tissues.

2. Mechanism of thyroid hormone action

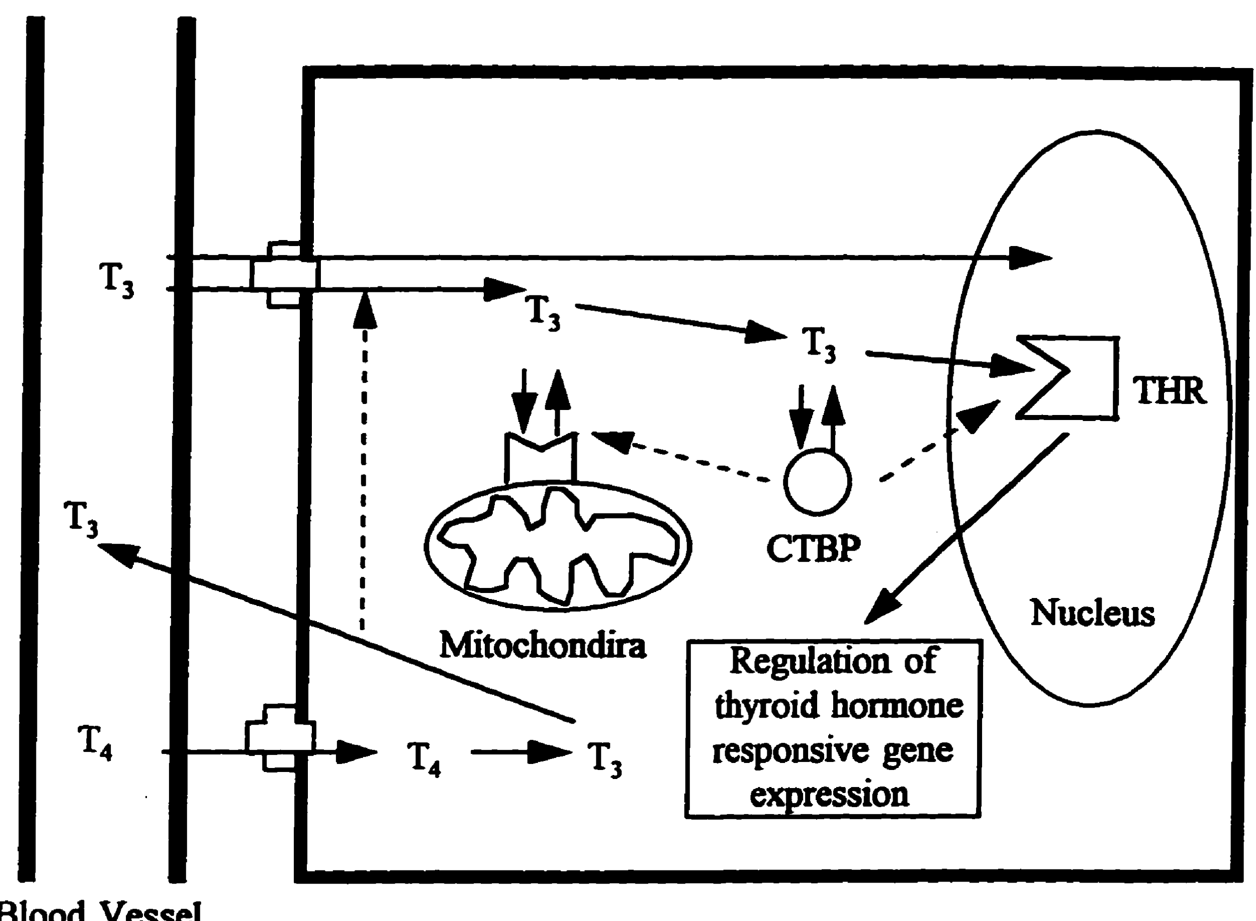
Thyroid hormone actions are mediated by interacting with specific receptors in the nucleus and mitochondria (Oppenheimer *et al.*, 1987; Sterling *et al.*, 1978). After binding to these receptors, thyroid hormones control the accumulation of specific mRNA molecules that code for the synthesis of specific proteins regulated by the hormone. The best studied example of this process is the regulation of malic enzyme, which catalyzes the decarboxylation of malate to produce pyruvate and carbon dioxide. In the liver, T_3 induces an increase in malic enzyme production. Using cDNA probes, it was demostrated that the effect of T_3 is pre-translational and T_3 induces an 11-14 fold increase in malic enzyme mRNA content (Winberry *et al.*, 1983; Back *et al.*, 1986). T_3 also increased malic enzyme mRNA content in heart and kidney (Nikodem *et al.*, 1989).

Initially, thyroid hormones are taken up by thyroid hormone responsive cells by endocytosis after binding to cell surface proteins (Halpen and Hinkle, 1982). Energy

dependent high and low affinity thyroid hormone uptake systems exist on the cell membrane (Krenning *et al.*, 1980; Gharbi and Torresani, 1979). It remains unclear as to whether T_3 and T_4 are taken up through the same carriers and this may depend on the type of tissue since hepatocytes appear to use different carriers whereas, the same carrier appears to be used within the pituitary (Krenning *et al.*, 1981; Everts *et al.*, 1994). Within the cell, T_4 is deiodinated to form T_3. T_3 may act at different locations within the cell (Fig. 10). T_3 binding to sites at the mitochondria are known to stimulate mitochondrial oxidatitve phosphorylation within two minutes of its addition (Sterling *et al.*, 1977). Within the cytosol, T_3 may bind to the cytosolic binding protein, which is a 76 kDa protein (Kobayashi *et al.*, 1991). This protein may control the intracellular reservoir of hormones, since it has been proposed to function in the transfer of thyroid hormones to the mitochondria (Hashizume *et al.*, 1986), but not the nucleus (Hashizume *et al.*, 1987). It is unknown how T_3 is transported into the nucleus, but most thyroid hormone biological activity is exerted within the nucleus where T_3 binds to the thyroid hormone receptor. The nuclear thyroid hormone receptor is an acidic non-histone protein with a molecular weight of 47-57 kDa (Ichikawa and De Groot, 1987). This receptor binds tightly to T_3 and specific DNA sequences, termed thyroid hormone response elements, and is distributed in transcriptionally active chromatin (Samuels *et al.*, 1977).

A model has been developed for the thyroid hormone regulation of malic enzyme gene expression (Fig. 11). Thyroid hormone receptor dimers can bind to the malic enzyme thyroid hormone response element and inhibit transcription (Petty *et al.*, 1990). Upon T_3 binding to its receptor, the receptor undergoes a conformational change that makes the surface charges of the thyroid hormone receptor less hydrophobic (Ichikawa *et al.*, 1988). This change, causes the protein-protein interaction of the dimer to be disturbed and the destabilized receptor is released from the thyroid hormone response element as

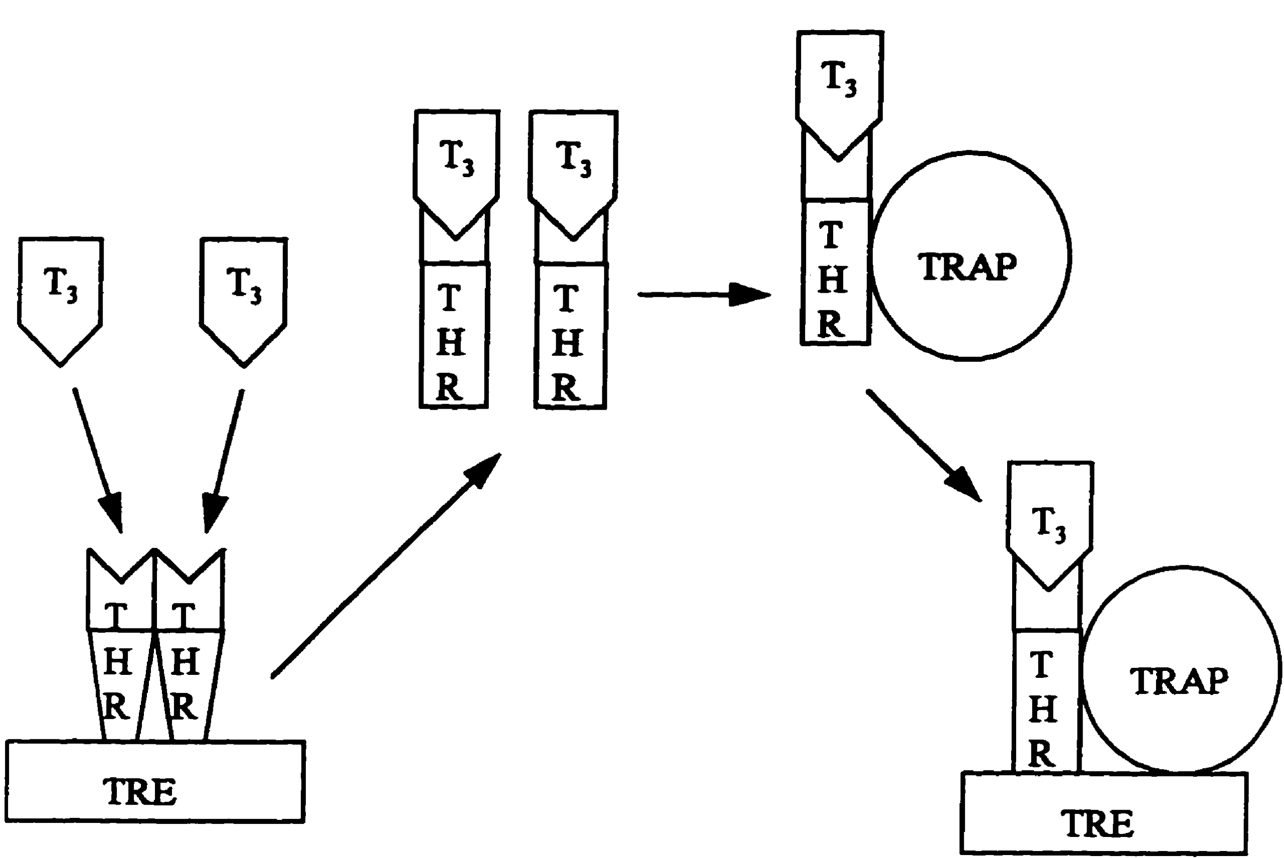
Figure 10



Thyroid hormone action in the cell.

T_4 , L-thyroxine; T_3 3,5,3'-triidodthyronine;
THR, nuclear thyroid hormone receptor;
CTBP, cytosolic thyroid hormone binding protein

Figure 11



Model for the regulation malic enzyme gene expression by 3,5,3'-triiodothyronine

T_3, 3,5,3'-triiodothyronine; THR, nuclear thyroid hormone receptor; TRE, thyroid hormone response element; TRAP, thyroid hormone receptor auxiliary protein.

monomers with T_3 bound (Yen *et al.*, 1992). Thyroid hormone receptor monomers with T_3 bound may bind to another protein called the thyroid hormone receptor auxiliary protein, which stabilizes the interaction of the thyroid hormone receptor with the thyroid hormone response element (Miyamoto *et al.*, 1993). This stable complex can increase the transcription of the malic enzyme gene in thyroid hormone responsive tissues.

3. Effect of Thyroid Hormones on the Heart.

The heart is a target of thyroid hormone action. Thyroid hormones are necessary for normal cardiac muscle growth. The thyroid state has a profound effect on cardiac mechanical properties. Hyperthyroidism increases myocardial contractility and oxygen demand. Hyperthyroidism also causes tachycardia, increases symptoms of angina and may lead to congestive heart failure (Aronow, 1995). Hypothyroidism decreases myocardial contractility and causes bradycardia. Thyroid hormones also have significant effects upon cardiac metabolic properties. Treatment with thyroid hormone increases the rate of protein turnover and the sensitivity to other hormones. For example, hyperthyroidism increases and hypothyroidism decreases the number of β-adrenergic receptors in the heart and consequently the sensitivity to catecholamines is dependent upon the thyroid state of the animal (Tsai and Chen, 1978). The thyroid state also affects heart size and alters mitochondrial oxidative phosphorylation (Aronow, 1995).

4. Thyroid hormone regulation of phospholipid metabolism

Changes in cellular lipid composition and metabolism are only one of the diverse effects of altered thyroid states. It is well recognized that thyroid hormones do regulate a number of lipid metabolizing enzymes (Hoch, 1988). For example, heart microsomal acyl-CoA:glycerol-3-phosphate acyltransferase (Dang *et al.*, 1985) as well as heart microsomal and lysosomal phosphatidate phosphohydrolase activity are regulated by thyroid hormones (Kako and Patterson, 1975; Kako and Liu, 1974).

i. Effect of thyroid hormones on phospholipid composition

The thyroid state of an animal influences the phospholipid content of certain thyroid responsive tissues. The first example of thyroid hormones affecting phospholipid content showed that *in vivo* T_4 administration to rats increased the total phospholipid content of the rat liver compared to controls (Ellefson and Mason, 1963). Later, increased incorporation of $^{32}P_i$ into most rat liver mitochondrial phospholipids (including PE) was observed in T_4 treated rats, compared to controls (Nelson and Cornatzer, 1965). Likewise, $^{32}P_i$ incorporation into PC and PE of rat heart mitochondria was increased in T_4 treated rats compared with controls. Hearts from rats treated for 30 days with T_3 had increased PE, PC and CL lipid phosphorus compared to controls, as well as increased ^{14}C-palmitic acid (16:0) incorporation into these same phospholipids (Vasev *et al.*, 1977). Hyperthyroidism induced by T_3 treatment of rats increased the CL content of heart mitochondria compared to controls without affecting the content of other phospholipids (Paradies *et al.*, 1994). Recently it was shown that hyperthyroidism induced by T_4 treatment of rats increased the CL and PG content in heart mitochondria (Cao *et al.*, 1995). Treatment of rats with either T_3 or T_4 increased the PE content and decreased the PC content in rat brain mitochondria (Bangur *et al.*, 1995). These changes were significant enough to cause an increase in mitochondrial membrane fluidity. Liver mitochondria from hypothyroid rats had double the amount of CL, whereas PC content was slightly decreased and PE content unchanged, compared with controls (Hoch *et al.*, 1981). However, later experiments showed that hypothyroid rats had increased PE and decreased PC content in liver mitochondria (Ruggiero *et al.*, 1984). In heart mitochondria from hypothyroid rats, the CL and PE content was decreased and the PC content was increased compared to controls (Paradies and Ruggiero, 1989). However, later experiments showed that in heart mitochondria from hypothyroid rats, only the CL content

significantly decreased compared to the controls (Paradies *et al.*, 1993). T_3 treatment of these hypothyroid rats returned the mitochondrial CL content to the control level.

ii. Effect of thyroid hormones on phospholipid fatty acid composition

The thyroid state of an animal influences the phospholipid fatty acid composition. For example, the stearic acid (18:0) content was increased and the linoleic acid (18:2) content decreased in cardiac mitochondria from T_4 treated rats compared with controls (Steffen and Platner, 1976). In cardiac microsomes, the palmitic (16:0) and stearic (18:0) acid contents were increased and the linoleic (18:2) and arachidonic (20:4) acid contents were decreased in the T_4 treated rats compared with the controls (Steffen and Platner, 1976). In the microsomes of livers from hyperthyroid rats treated with T_3, an increased stearic (18:0) and arachidonic (20:4) acid content and decreased palmitic (16:0) and linoleic (18:2) acid content, compared to controls was observed (Faas and Carter, 1981). Compared with controls, linoleic (18:2) and arachidonic (20:4) acid content increased and palmitic (16:0) and oleic (18:1) acid content decreased in heart sarcolemmma from hyperthyroid rabbits (Szymanska *et al.*, 1991). Linoleic acid (18:2) content was increased and the arachidonic acid (20:4) content was decreased in liver mitochondria and microsomes of hypothyroid rats compared to controls (Hoch *et al.*, 1981). This observation suggested that the conversion of linoleic acid to arachidonic acid is defective in the livers of hypothyroid animals. The palmitic (16:0) and arachidonic (20:4) acid contents were increased whereas the stearic (18:0) and linoleic (18:2) acid contents were decreased in the mitochondria of hypothyroid rats compared to controls (Paradies and Ruggiero, 1989). A two-fold increase in the linoleic acid (18:2) content and a decrease in the arachidonic acid (20:4) content of mitochondrial PE in liver from hypothyroid rats was observed (Raederstorff *et al.*, 1991). Compared with controls, palmitic (16:0) and

linoleic (18:2) acid content increased and arachidonic acid (20:4) decerased in mitochondrial PC from livers of hypothyroid rats (Raederstorff *et al.*, 1991).

Alterations in the fatty acid composition of membrane phospholipids by thyroid hormones may have important physiological consequences. Thyroid hormones can alter membrane fluidity by altering the unsaturation of membrane phospholipid fatty acid composition. This may be the mechanism whereby the activity of some membrane associated enzymes are altered by thyroid hormones (Hulbert, 1978).

RESEARCH AIMS

Thyroid hormones control the activity of many lipid metabolizing enzymes in the heart (Hoch, 1988). For example, it has been recently demonstrated that in hearts from hyperthyroid rats there is a significant increase in the phosphatidylglycerol content due to an increase in the activity of phosphatidylglycerolphosphate synthase (Cao *et al.*, 1995). The regulatory mechanisms which control phospholipid remodeling in the heart are largely unknown. Given that thyroid hormones have such profound effects on lipid metabolizing enzymes in the heart, it is possible that they may also regulate the remodeling of certain phospholipids. The regulation of phospholipid remodeling is important to prevent the accumulation of membrane perturbing lysophospholipids (Zubay, 1988). Furthermore, it is important for the establishment of the appropriate tissue specific phospholipid fatty acid composition (Holub and Kuksis, 1978). Therefore the objective of this study is to investigate whether the remodeling of a major cardiac phospholipid is regulated by thyroid hormones in the heart.

METHODS AND MATERIALS

I. Materials

1. Experimental Animals

Male Sprague Dawley rats (150-175g body weight) were used throughout the study. Rats were maintained on Purina rat Chow and tap water *ad libitum*, in a light and temperature controlled room. Treatment of animals conformed to the Guidelines of the Canadian Council on Animal Care.

2. Chemicals

[1-^{14}C]arachidonic acid, [1-^{14}C]linoleic acid, [1-^{14}C]palmitic acid, [1-^{14}C]oleic acid and [1-^{14}C]oleoyl-CoA, [1,2-^{14}C]ethanolamine and [^{3}H(G)]serine was obtained from Mandel Scientific Co., Guelph, Ontario,. Ecolite scintillation cocktail was obtained from ICN Biochemicals, Thin layer plates (silica gel 60, 0.25 mm thickness) were obtained from VWR-CanLab, Winnipeg, Manitoba, Canada. Sucrose, ATP, bovine serum albumin, palmitic acid, 6-*n*-propyl-2-thiouracil (PTU), thyroxine (T_4) and Trizma base were purchased from Sigma Chemical Co., St. Louis, Missouri. Oleoyl-CoA, egg LPE, egg LPC, arachidonic acid, linoleic acid, and oleic acid were obtained from Serdary Research Laboratories , Englewood Cliffs, New Jersey. All other chemicals and solvents were of analytical grade, obtained from either Fisher Scientific, Edmonton, Alberta, Canada; Sigma Chemical Co., St. Louis, Missouri; or VWR-CanLab, Mississauga, Ontario, Canada.

II. Methods

1. Preparation of animals

i. Hyperthyroid animals

Rats were daily injected intraperitoneally for 5 days with 250 μg/ Kg T_4 dissolved in 0.9% NaCl. Controls received only 0.9 % NaCl. After an overnight fast on the sixth day the animals were sacrificed.

ii. Hypothyroid animals

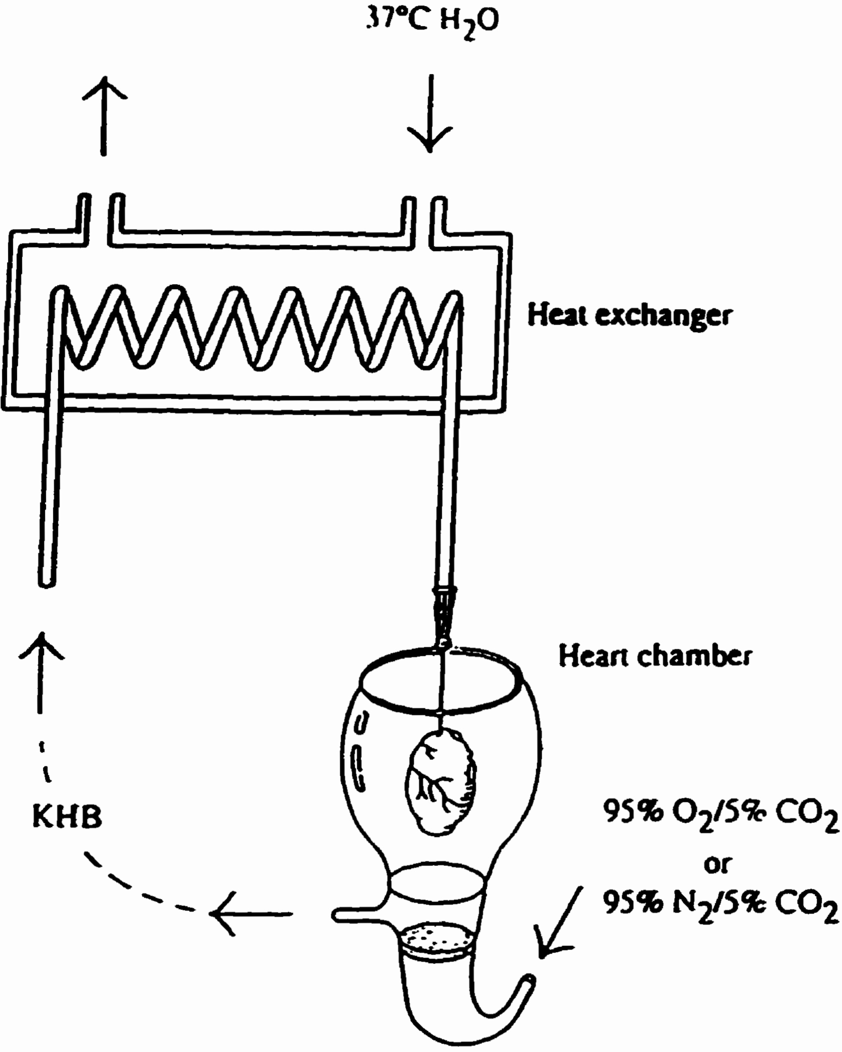
Rats were administered PTU (0.05 % w/v) in their drinking water *ad libitum* for six days. Controls received only the drinking water *ad libitum* for the same period. After an overnight fast on the sixth day the animals were sacrificed.

2. Heart Tissue Preparations

i. Perfusion of isolated rat hearts in Langendorff mode

Male Sprague Dawley rats were sacrificed by decapitation. The heart was quickly removed and cannulated via the aorta using a modified syringe needle (18 gauge). The remaining blood in the coronary circulation was removed by injecting the heart with 5 mL of Krebs Henseleit buffer (Krebs and Henseleit, 1932) using a 10 cc syringe. Buffer was prepared fresh by combining 100 mL of solution A (contained 70.1 g/L sodium chloride, 21.0 g/L sodium bicarbonate and 9.91 g/L dextrose), 10 mL of solution B (contained 3.55 g/100 mL potassium chloride, 2.94 g/100 mL magnesium sulfate, and 1.63 g/100 mL sodium phosphate, monobasic), 5 mL of solution C (contained 3.37 g/100 mL calcium chloride, dihydrate), and double distilled water was added to a final volume of 1 L in a volumetric flask. Immediately after, the heart was placed on the perfusion apparatus (Fig.

Figure 12



Retrograde perfusion apparatus.

Krebs Henseleit buffer flows through the heat exchanger and is warmed to 37 °C. The perfusate flows into the aorta in a retrograde manner. The buffer is collected in the heart chamber and recirculated. The buffer in the heart chamber is saturated with 95% oxygen/ 5% carbon dioxide.

12) and perfused in the Langendorff mode (Langendorff, 1895) for 10 min to allow for stabilization. After which, 12.5 mL of Krebs Henseleit buffer containing either radioactive or non-radioactive compounds at various time intervals was perfused through the heart. All perfusions were performed at 37 °C with a flow rate of 2.5 mL/min. The viability of the heart under these conditions could be maintained for up to 4 h of perfusion (Arthur and Choy, 1984). Subsequent to perfusion, 10 mL of air was injected into the heart to remove the residual radioactive or non-radioactive perfusate that remained in the coronary circulation and the hearts frozen in liquid nitrogen. The hearts were freeze dried overnight.

ii. Radioactive labeling studies studies in the isolated perfused heart.

To study the effect of thyroid state on phospholipid remodeling in the isolated perfused rat heart, hearts were perfused for 30 min with 12.5 mL containing 0.01-0.1 mM [1-^{14}C] arachidonic, palmitic, linoleic and oleic acids (0.4 µCi/mL) bound to bovine serum albumin in a 1:1 molar ratio. The concentrations of fatty acids used throughout the experiments were representative of the physiological plasma concentration in humans (Shimomura *et al.*, 1986). In the experiments to determine the effect of the thyroid state on biosynthesis in the isolated perfused rat heart, hearts were perfused for 30 min. with 12.5 mL Krebs Henseleit buffer containing [1,2-^{14}C]ethanolamine and L-[^{3}H(G)]serine (0.4 µCi/mL). The perfusate was saturated with 95% O_2 / 5% CO_2.

iii. Sub-cellular fractionation by differential centrifugation.

Crude sub-cellular fractions were prepared for assay of enzyme activities. The animal was sacrificed by decapitation and the heart was quickly removed and blood remaining in the coronary circulation was removed using 30 cc of homogenization buffer containing 10 mM Tris-HCl, 0.25 M sucrose, 2 mM EDTA, pH 7.4. A 10% homogenate was prepared by homogenizing the heart (Polytron 20 sec burst) in homogenizing buffer.

The homogenate was centrifuged at 1000 X g for 20 min (Beckman J2-H refrigerated centrifuge with JA-20 rotor). The resulting pellet was discarded and the supernatant was centrifuged at 12 000 X g for 10 min. The resulting pellet was resuspended in 5 mL of homogenizing buffer by 15 strokes of a loose fitting hand held dounce tissue homogenizer and again centrifuged at 12 000 X g for 10 min. The resulting pellet was resuspended in 1 mL homogenizing buffer with a tight fitting dounce tissue homogenizer. This was used as the source of the mitochondrial fraction for enzyme assay. The post-mitochondrial supernatant was centrifuged for 60 min at 100 000 X g (Beckman Ultra centrifuge with 70.1 Ti rotor). The resulting pellet was resuspended in 0.5 mL of homogenizing buffer by 15 strokes of a tight fitting hand held dounce tissue homogenizer and used as the source of the microsomal fraction. The supernatant was used as the source of the cytosolic fraction. All subcellular fractions were aliquotted into 1.5 mL microfuge tubes and frozen. Marker enzyme analysis revealed that the mitochondrial fraction was contaminated with 10% microsomal particles and the microsomal fraction with 5% mitochondrial particles (Hatch and Choy, 1987).

3. Preparation and Isolation of Lipids

i. Lipid extraction

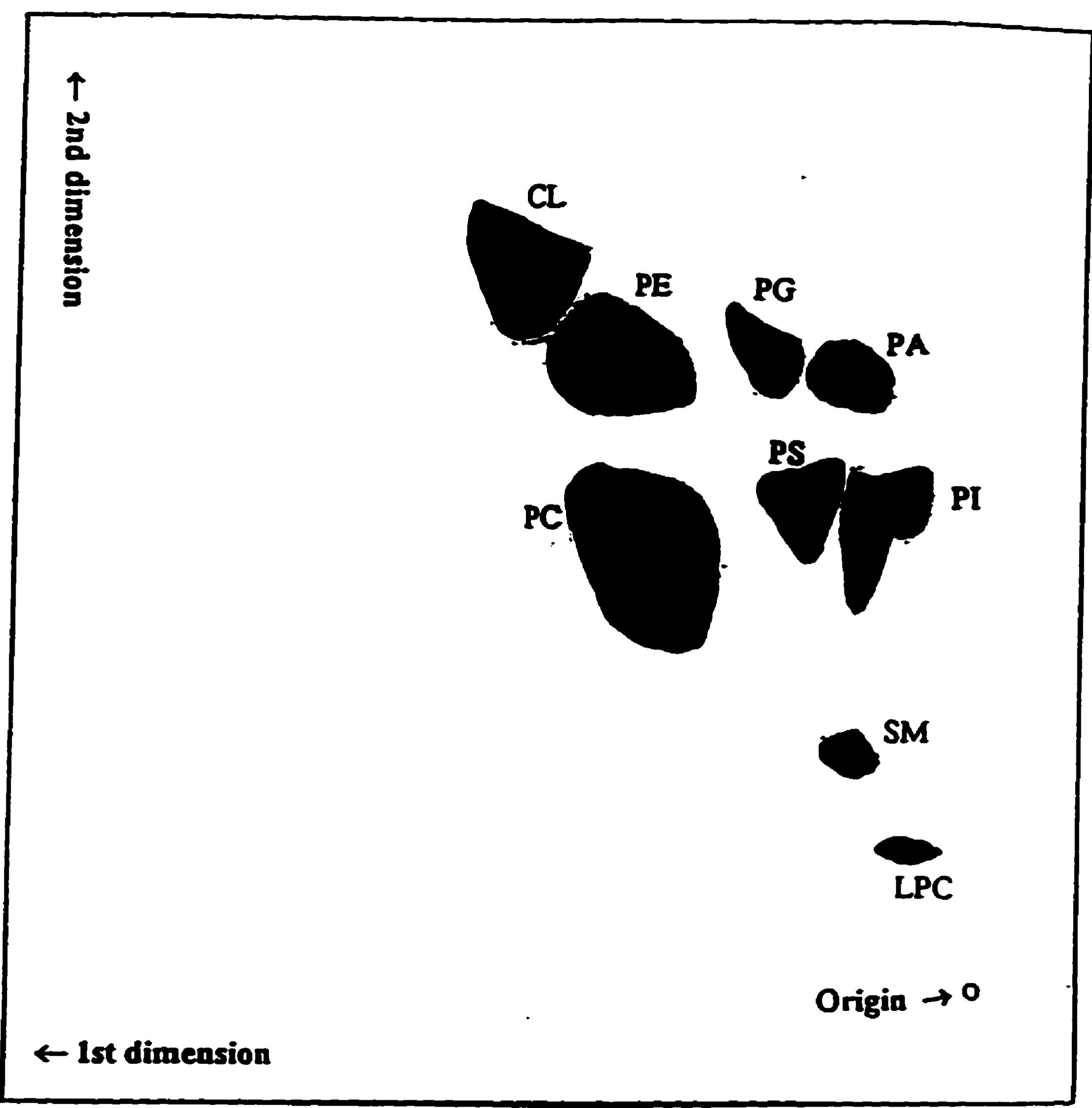
Subsequent to perfusion, freeze dried hearts were weighed (typically 0.07-0.2 g) and homogenized by a 20 sec burst of a Polytron Homogenizer (Kinematika, Lucerne, Switzerland) in 5 mL of chloroform:methanol (2:1 v/v). Tissue remaining on the homogenizer probe was removed by homogenization with another 5 mL of chloroform:methanol (2:1 v/v). The two 5 mL homogenates were combined and centrifuged at full speed for 20 min in a bench top centrifuge (Model HN-SII, Fisher Scientific, Edmonton, Alberta) to pellet debris. After centrifugation, the homogenate was decanted into a 16 X 125 mm screw cap tube. Addition of 5 mL of 0.73% sodium

chloride to the homogenate resulted in the formation of organic and aqueous phases. The tube was centrifuged at full speed for 10 min in a bench top centrifuge causing biphasic separation. The upper aqueous phase was removed into separate test tubes for separation of aqueous containing metabolites and determination of radioactivity associated with the aqueous phase. The remaining organic phase was further washed with 5 mL of theoretical upper phase (chloroform:methanol:0.9% sodium chloride, 3:48:47 v/v). The tube was centrifuged at full speed for 10 min in a bench top centrifuge. The upper aqueous phase was removed and combined with the previous upper phase. The remaining organic phase was dried down under a stream of nitrogen gas.

ii. Isolation of lipids by thin-layer chromatography.

For lipid samples obtained from perfused rat hearts, 20 X 20 cm thin layer chromatography plates treated with 0.4 M boric acid and dried were used to resolve individual phospholipid species. Plates were sprayed with a 0.4 M solution of boric acid overnight (Poorthius *et al.*, 1976). Before application of the lipid sample, the thin layer plates were heat activated for 1 h at 145 ^{o}C. The thin layer plate was allowed to cool before the sample was applied. The dried organic sample was resuspended in 100 µL of chloroform:methanol (2:1 v/v) and 25 µL of the resuspended lipid sample was applied to the plate. In addition, a 5 µL aliquot of the sample was taken for determination of total radioactivity associated with the sample. The thin layer plate was subjected to a two-dimensional solvent system consisting of chloroform:methanol:ammonium hydroxide:water (70:30:2:3 v/v) in the first dimension and at 90° to the first, the second dimension consisted of chloroform:methanol:water (65:35:5 v/v). The thin-layer plate was allowed to dry between dimensions. The separated phospholipids were visualized by exposing the plate to iodine vapor. The two dimensional solvent system has a characteristic phospholipid pattern (Fig. 13). Radioactive phospholipids were removed

Figure 13



Two dimensional thin layer chromatogram of rat heart phospholipids.

Abbreviations used: CL, cardiolipin; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI phosphatidylinositol; PS, phosphatidylserine; SM, sphingomyelin; LPC, lysophosphatidylcholine.

into 6 mL scintillation vials and the radioactivity incorporated phospholipids was determined in a Beckman Model LS 3801 liquid scintillation counter.

For the determination of radioactive neutral lipids, 15 μL of the resuspended lipid sample was applied to the plate. Neutral lipids were resolved using a one-dimensional solvent system consisting of petroleum ether:diethyl ether:acetic acid (80:20:1 v/v). Radioactive neutral lipids were visualized in iodine vapor, removed and the radioactivity determined as described previously.

iii. Analysis of radioactivity in ethanolamine containing metabolites.

An aliquot of the aqueous phase was dried under air and resuspended in 1 mL of water. 100 μL of the aqueous sample was spotted on the plate and the ethanolamine containing metabolites were separated by a one dimensional thin-layer chromatography with a solvent containing ethanol:2% ammonium hydroxide (1:2 v/v) (Sundler and Akesson, 1975). Ethanolamine containing metabolites were visualized by spraying the chromatogram with ninhydrin solution. Ethanolamine, CDP-ethanolamine, and phosphoethanolamine were removed from the chromatogram and the radioactivity determined by scintillation counting.

4. Gas chromatography

i. Elution of lipids from silica gel.

Phospholipids and neutral lipids were eluted from silica gel according to the method of Arvidson (1968). Lipids samples separated as described previously were scraped from the thin layer chromatography plate into screw capped tubes and stored in 3 mL of chloroform:methanol:acetic acid:water (50:39:1:10 v/v) under nitrogen overnight in the freezer. The following day, the silica gel was eluted 3 times with 3 mL of the chloroform:methanol:acetic acid:water (50:39:1:10 v/v). The eluate was pooled in clean screw capped tubes and 1 mL water was added. The tubes were centrifuged at full speed

in a bench top centrifuge to separate the aqueous and organic phases. The upper aqueous layer was discarded. The lower organic layer was washed twice with 2 mL methanol:water (1:1 v/v), and the aqueous layer was discarded after each wash. Samples were stored under nitrogen in the freezer.

ii. Preparation of fatty acid methyl esters

Fatty acid methyl esters were prepared according to the method of Tardi *et al.* (1992). Lipid samples were dried under nitrogen. Subsequently, 0.5 mL BF_3:methanol reagent was added to the screw capped tube, mixed and sonicated briefly to dissolve the lipids. The screw capped tubes were covered with teflon tape and capped before being placed in a 100 ^{o}C water bath for 5 min. Afterwards the tubes were cooled in ice and 0.5 mL of water was added. Tubes were shaken well and 1.5 mL of petroleum ether was added and the tubes were mixed thoroughly. Phases were separated by centrifugation for 2 min in the bench top centrifuge. The upper phase was removed into a clean screw cap tube and another 1.5 mL of petroleum ether was added to the lower phase, mixed and centrifuged as described previously. Afterwards, the upper phase was removed and combined with the first. The pooled upper phase was washed twice with 1.5 mL of water, centrifuged and the lower phase was discarded each time. The upper phases were dried under nitrogen and dissolved in 50-100 µL of heptane prior to injection into the gas chromatograph. Typically, 10 µL of sample was injected into the Shimadzu GC-14A gas chromatograph using a 10 µL Hamilton syringe.

5. Analytical Methods and Assays

i. Determination of lipid phosphorous

Phospholipid phosphorous was determined according to the method of Rouser *et al.* (1966). Phospholipid silica gel samples scraped from thin layer chromatography plates were placed in clean test tubes as well as 5 µL of the total organic sample from the heart.

Potassium phosphate (monobasic) standards ranging from 0-200 nmoles were also prepared at the same time. Then 450 µL of perchloric acid (70% v/v) was added to each tube, then tubes were covered with a piece of glass and heated at 180 °C for 90 min in the heater blocks. After allowing to cool, 2.5 mL of water was added to each tube. Subsequently, 0.5 mL of ammonium molybdate (2.5 % w/v) and 0.5 mL ascorbic acid (10% w/v) were added to each tube. Tubes were then covered with aluminum foil and incubated in hot 95 °C water for 15 min. After the samples were cooled and centrifuged for 15 min at full speed in the bench top centrifuge, absorbances were immediately read at 820 nm in a Milton Roy spectronic 1001 plus spectrophotometer. The values were then converted to g of phosphorous using a factor derived from the standard curve prepared using the potassium phosphate (monobasic).

ii. Determination of protein

Protein concentration of the crude mitochondrial, microsomal and cytosolic fractions were determined by a modified method of Bradford (Bradford, 1976). A 5 µL aliquot of the crude fraction was incubated with 5 mL of water:Bio Rad Protein Dye Reagent (4:1 v/v). The mixture was vortexed and incubated at room temperature for 5 min. Absorbance was measure at 595 nm against a blank. Fatty acid free bovine serum albumin (1 mg/mL) was used as a standard.

iii. Assay of 1-acylglycerophosphocholine acyltransferase

Acyl-CoA:1-acylglycerophosphocholine acyltransferase activity was determined in rat heart microsomal fraction. The assay mixture contained 75 mM Tris-HCl, pH 8.5, 150 nmole of LPC (egg), 64.4 µmole of oleoyl-CoA and 50 µg of protein from the crude sub-cellular fraction in a total volume of 0.7 mL. Labeled [1-^{14}C]oleoyl-CoA (90,000 dpm/tube) was used in the assay and its addition initiated the reaction. Incubation was for 30 min at 25 °C in a shaking water bath. The reaction was stopped by the addition of 3

mL of chloroform:methanol (2:1 v/v). This was followed by the addition of 0.8 mL potassium chloride (0.9 % w/v) to facilitate phase separation and the tubes were vortexed and centrifuged at full speed in a bench top centrifuge. The aqueous fraction was removed by suction and the organic fraction was dried under nitrogen gas. Small 10 X 10 cm 0.4 M boric acid treated thin layer chromatography plates were used to resolve the individual phospholipid species. Before application of the lipid sample, the thin layer plates were heat activated, as before. The dried organic sample was resuspended in 25 µL of chloroform:methanol (2:1 v/v) and 20 µL of the resuspended lipid sample was applied to the plate. The plate was subjected to the same two-dimensional solvent system and the radioactivity associated with phospholipids of interest were determined as described previously (II.3.ii.).

iv. Assay of 1-acylglycerophospholethanolamine acyltransferase

Acyl-CoA:1-acylglycerophosphoethanolamine acyltransferase activity was determined in rat heart microsomal, mitochondrial and cytosolic fractions according to the optimum conditions determined by Arthur *et al.* (1987a). The assay mixture contained 75 mM Tris-HCl, pH 8.5, 150 nmole of LPE (egg), 64.4 µmole of oleoyl-CoA and 50 µg of protein from crude sub-cellular fraction in a total volume of 0.7 mL. Radioactive [1-^{14}C]oleoyl-CoA (90,000 dpm/tube) was used in the assay and its addition initiated the reaction. Incubation was for 30 min at 25 ^{o}C in a shaking water bath. The reaction was stopped by the addition of 3 mL of chloroform:methanol (2:1 v/v) and then 0.8 mL potassium chloride (0.9% w/v) was added to facilitate phase separation. The aqueous fraction was removed by suction and the organic fraction dried under nitrogen gas. Individual radioactive phospholipids were separated and radioactivity associated with these was determined in the same manner as described for acyl-CoA:1-acylglycerphosphocholine acyltransferase.

v. Assay of long chain fatty acyl-CoA synthetase

Long chain fatty acyl-CoA synthetase activity was determined as described by Koshlukova *et al.* (1992). The assay mixture contained 0.1 M Tris-HCl, pH 8.0, 1.6 mM Triton X-100, 5 mM dithiothreitol, 150 mM potassium chloride, 15 mM magnesium chloride, 10 mM ATP, 0.6 mM CoA and 50 μg of protein from crude microsomal and mitochondrial fractions in a total volume of 0.2 mL. Radioactive [1-^{14}C]oleic acid (20,000 dpm/tube) was used in the assay and its addition initiated the reaction. Incubation was for 5 min at 37 ^{o}C. The reaction was terminated by the addition of 2.25 mL of isopropanol:hexane:1M sulfuric acid (40:10:1 v/v). Then 1.5 mL hexane and 1 mL water were added to each sample and the upper phase was removed. The lower phase was washed twice to remove the unesterified fatty acids. The radioactivity of the water soluble product, labeled [1-^{14}C]oleoyl-CoA was counted on a Beckman Model LS 3801 liquid scintillation counter.

vi. Assay of phospholipase A

Phospholipase A activity was determined as described by Cao and Hatch (1995). The assay mixture contained 50 mM Tris-HCl, pH 8.5, 5 mM calcium chloride and 100 μg of protein from crude microsomal, mitochondrial and cytosolic fractions in a total volume of 0.5 mL. In some experiments radioactive phosphatidyl[1,2-^{14}C]ethanolamine (90,000 dpm/tube) was used as the substrate and in others [1-^{14}C]1-palmitoyl-2-palmitoyl-phosphatidylcholine (90,000 dpm/tube) was used. The reaction was initiated by the addition of radioactive substrate and incubated at 37 ^{o}C for 30 min. The reaction was terminated by the addition of 2 mL of chloroform followed by the addition of 1 mL 0.1 M HCl in methanol. To facilitate phase separation, 1 mL of NaCl (0.75 % w/v) was added

and the mixture was centrifuged at full speed in a bench top centrifuge. The aqueous phase was removed by suction and the organic phase was dried under nitrogen gas. Thin layer chromatography plates treated with ammonium sulfate (0.4% w/v) were used. The thin layer plates were heat activated, as before and the dried organic sample was resuspended in 25 µL of chloroform:methanol (2:1 v/v) and 20 µL of the resuspended lipid sample was applied to the plate. The thin layer plate was developed in a solvent system containing chloroform:methanol:acetic acid:acetone:water (40:25:7:4:2 v/v). The plate was stained with iodine vapor and silica gel corresponding to $[^{14}C]$lysophosphatidyl -choline or -ethanolamine was removed and the radioactivity determined by a Beckman Model LS 3801 liquid scintillation counter with internal standard.

vii. Statistical determination

All results are depicted as mean standard deviation (number of experiments) unless otherwise indicated. Student's t-test was used for the determination of statistical significance. The level of significance was defined as $P < 0.025$.

RESULTS

I. Effect of thyroid condition on rats.

Rats were rendered hyperthyroid by daily injections of 250 μg/ Kg body weight of thyroxine dissolved in saline, for five days. Rats were rendered hypothyroid by receiving 0.05% 6-*n*-propyl-2-thiouracil *ad libitum* in their drinking water for 5 days. Control rats were injected with saline only. The body weight of the rats was not significantly affected by their thyroid condition (Table VII). However, hyperthyroidism produced a 30% ($P < 0.025$) increase in heart ventricular weight, compared with controls. Hypothyroidism produced a 30% ($P < 0.025$) decrease in the heart ventricular weight. In addition, the ratio of heart weight /body weight (X 1000) was increased from 0.69 in controls to 0.93 ($P < 0.025$) in hyperthyroid animals and decreased to 0.53 ($P < 0.025$) in hypothyroid animals. Therefore, the hearts from rats used in this study exhibited the characteristic properties of hyperthyroidism and hypothyroidism in this species.

II. Effect of thyroid condition on incorporation of radiolabeled fatty acids into the lipids of the heart.

To determine whether the incorporation of radiolabeled fatty acids into cardiac neutral and phospholipids was affected by the thyroid condition, rat hearts were perfused in Langendorff mode (Langendorff, 1895) (Fig. 12) for 30 min in a continuous pulse labeling experiment. The perfusate contained [1-^{14}C] long chain fatty acids bound to albumin in a 1:1 molar ratio at concentrations that were physiologically normal in the blood serum (Shimomura *et al.*, 1986). The fatty acids used in this study included palmitic acid (16:0), oleic acid (18:1), linoleic acid (18:2) and arachidonic acid (20:4). These were selected as being representative of the major fatty acids presented to the heart in blood serum. Subsequent to perfusion, hearts were immediately frozen in liquid nitrogen and freeze dried. Then hearts were homogenized in 10 mL of chloroform:methanol (2:1 v/v).

Table VII

Effect of thyroid condition on rat body weight, ventricular weight and ventricular/body weight ratio (X 1000)

Animals were treated as described in Methods II. 1. Data represents the mean ± standard deviation of 9 hearts. * P < 0.025.

	Freeze-dried heart weight g	Rat Weight g	Heart/Body Weight X 1000
Control	0.142 ± 0.012	206.3 ± 17.3	0.69 ± 0.05
Hyperthyroid	0.184 ± 0.023 *	198.0 ± 10.2	0.93 ± 0.08*
Hypothyroid	0.099 ± 0.009 *	187.8 ± 8.9	0.53 ± 0.05*

Total radioactivity incorporated into the organic fraction of the hearts was not significantly affected by the thyroid condition for all the fatty acids examined (Tables XVIII-XXI).

1. Effect of thyroid condition on incorporation of 0.1 mM [1-^{14}C] palmitic acid into individual neutral and phospholipids in the perfused rat heart.

The labeling of PE by [1-^{14}C] palmitic acid in the perfused rat heart was affected by the thyroid condition (Table XII). In hyperthyroid animals, the labeling of PE was significantly increased by 2 fold ($P < 0.025$) compared with controls (Table XII). Hypothyroidism increased the labeling of PE by 4 fold compared with controls, however this change was not statistically significant ($P = 0.04$). The labeling of all other cardiac phospholipids by [1-^{14}C] palmitic acid was not significantly affected by the thyroid state. However, the labeling of TG by [1-^{14}C] palmitic acid in hearts from hyperthyroid animals was significantly decreased by 75% ($P < 0.025$) compared with controls. Hypothyroidism also decreased the labeling of cardiac TG by 66% compared with controls, however this change was not statistically significant ($P = 0.05$). Labeling of DG was not significantly affected by the thyroid condition.

2. Effect of thyroid condition on incorporation of 0.1 mM [1-^{14}C] oleic acid into individual neutral and phospholipids in the perfused rat heart.

In hyperthyroid animals, the labeling of all neutral and phospholipids by [1-^{14}C] oleic acid in the perfused rat heart, with the notable exception of PE, was unchanged compared with controls (Table XIII). The labeling of PE was increased 1.5 fold ($P < 0.025$) compared with controls. Hypothyroidism did not significantly affect the labeling of PE by [1-^{14}C] oleic acid. However, hypothyroidism did significantly increase the labeling of PC 2 fold ($P < 0.025$) and PA 1.9 fold ($P < 0.025$) compared to controls. The labeling of all other cardiac phospholipids was not significantly affected by hypothyroidism. The

Table VIII

Effect of thyroid condition on incorporation of radioactivity into cardiac organic and aqueous fractions in hearts perfused in Landendorff mode for 30 min. with Krebs Henseleit buffer containing 0.1 mM [1-^{14}C]palmitic acid (16:0). Values represent the mean ± standard deviation of at least three hearts. * P < 0.025.

Thyroid Condition	Organic dpm X 10^6 /g freeze dried ventricle	Aqueous dpm X 10^6 /g freeze dried ventricle
Control	7.36 ± 1.61	0.54 ± 0.05
Hyperthyroid	4.05 ± 1.82	0.32 ± 0.13
Hypothyroid	4.97 ± 2.02	1.38 ± 0.31 *

Table IX

Effect of thyroid condition on incorporation of radioactivity into cardiac organic and aqueous fractions in hearts perfused for 30 min. in Langendorff mode with Krebs Henseleit buffer containing 0.1 mM [1-^{14}C]oleic acid (18:1). Values represent the mean ± standard deviation of at least three hearts.

Thyroid Condition	Organic dpm X 10^6 /g freeze dried ventricle	Aqueous dpm X 10^6 /g freeze dried ventricle
Control	13.6 ± 1.0	0.9 ± 0.1
Hyperthyroid	10.4 ± 0.3	0.6 ± 0.2
Hypothyroid	9.3 ± 3.7	0.9 ± 0.1

Table X

Effect of thyroid condition on incorporation of radioactivity into cardiac organic and aqueous fractions in hearts perfused for 30 min. in Langendorff mode with Krebs Henseleit buffer containing 0.08 mM [1-^{14}C]linoleic acid (18:2). Values represent the mean ± standard deviation of at least three hearts.

Thyroid Condition	Organic dpm/g freeze dried ventricle	Aqueous dpm/g freeze dried ventricle
Control	7.02 ± 0.51	0.52 ± 0.04
Hyperthyroid	3.40 ± 0.13	0.49 ± 0.07
Hypothyroid	6.42 ± 1.02	0.80 ± 0.32

Table XI

Effect of thyroid condition on incorporation of radioactivity into cardiac organic and aqueous freactions of hearts perfused in Langendorff mode for 30 min. with Krebs Henseleit buffer containing 0.01 mM [1-^{14}C]arachidonic acid (20:4). Values represent the mean ± standard deviation of at least three hearts.

Thyroid Condition	Organic dpm/g freeze dried ventricle	Aqueous dpm/g freeze dried ventricle
Control	11.63 ± 1.60	0.35 ± 0.03
Hyperthyroid	8.24 ± 3.93	0.34 ± 0.10
Hypothyroid	11.42 ± 1.32	0.60 ± 0.13

Table XII

Effect of thyroid condition on incorporation of radioactivity into cardiac neutral and phospholipids in rat hearts perfused in Langendorff mode for 30 min. with Krebs Henseleit buffer containing 0.1 mM [1-^{14}C] palmitic acid (16:0). Values represent the mean ± standard deviation of at least three hearts. * P < 0.025.

Lipid	Control	Hyperthyroid dpm X 10^5 /g freeze dried ventricle	Hypothyroid
Phosphatidylethanolamine	0.42 ± 0.08	0.89 ± 0.01 *	1.63 ± 0.47
Phosphatidylcholine	2.66 ± 0.17	1.98 ± 0.13	6.12 ± 2.37
Phosphatidylserine	0.08 ± 0.01	0.13 ± 0.04	0.13 ± 0.04
Phosphatidylinositol	0.77 ± 0.13	0.56 ± 0.17	1.02 ± 0.05
Phosphatidic Acid	1.02 ± 0.30	0.98 ± 0.12	1.06 ± 0.18
Cardiolipin	0.04 ± 0.01	0.07 ± 0.03	0.05 ± 0.01
Phosphatidylglycerol	0.15 ± 0.02	0.17 ± 0.03	0.21 ± 0.03
Lysophosphatidylcholine	0.06 ± 0.02	0.06 ± 0.01	0.06 ± 0.01
Diacylglycerol	7.90 ± 2.68	5.68 ± 2.38	6.38 ± 2.12
Triacylglycerol	62.06 ± 21.40	15.58 ± 7.83 *	21.22 ± 4.63

Table XIII

Effect of thyroid condition on incorporation of radioactivity into cardiac neutral and phospholipids in rat hearts perfused for 30 min. in Langendorff mode with Krebs Henseleit buffer containing 0.1 mM [1-^{14}C] oleic acid (18:1). Values represent the mean ± standard deviation of at least three hearts. * $P < 0.025$

Lipid	Control	Hyperthyroid dpm X 10^5 /g freeze dried ventricle	Hypothyroid
Phosphatidylethanolamine	2.08 ± 0.13	3.12 ± 0.57 *	2.21 ± 0.29
Phosphatidylcholine	3.12 ± 0.85	4.79 ± 0.05	6.19 ± 0.26 *
Phosphatidylserine	0.14 ± 0.02	0.21 ± 0.05	0.28 ± 0.06
Phosphatidylinositol	3.32 ± 0.82	2.67 ± 0.07	6.85 ± 1.35
Phosphatidic Acid	0.53 ± 0.12	0.80 ± 0.18	1.01 ± 0.19 *
Cardiolipin	0.14 ± 0.04	0.25 ± 0.07	0.16 ± 0.03
Phosphatidylglycerol	0.13 ± 0.05	0.15 ± 0.04	0.30 ± 0.10
Lysophosphatidylcholine	0.30 ± 0.07	0.17 ± 0.06	0.14 ± 0.03
Diacylglycerol	1.60 ± 0.06	1.76 ± 0.62	1.52 ± 0.65
Triacylglycerol	14.70 ± 4.02	20.28 ± 6.60	7.38 ± 3.68

labeling of cardiac neutral lipids by [1-^{14}C] oleic acid was not significantly affected by the thyroid condition.

3. Effect of thyroid condition on incorporation of 0.08 mM [1-^{14}C] linoleic acid into individual neutral and phospholipids in the perfused rat heart.

Among all phospholipids, only PE and CL labeling by [1-^{14}C] linoleic acid in the perfused rat heart were significantly affected by hyperthyroidism (Table XIV). In hyperthyroid animals, the labeling of PE was increased by 34% ($P < 0.025$) compared to controls. Hypothyroidism had little effect on the labeling of PE. The labeling of CL was increased 1.6 fold ($P < 0.025$) in hyperthyroid animals compared to controls. Compared to controls, hypothyroidism did not significantly affect the labeling of any of the phospholipids by [1-^{14}C] linoleic acid in the perfused rat heart. Labeling of cardiac DG was unaffected by the thyroid state of the animal. However, labeling of TG by [1-^{14}C] linoleic acid in the perfused rat heart was significantly decreased by 74% ($P < 0.025$) in hyperthyroid rats and increased by 1.4 fold ($P < 0.025$) in hypothyroid rats, compared to controls.

4. Effect of thyroid condition on incorporation of 0.01 mM [1-^{14}C] arachidonic acid into individual neutral and phospholipids in the perfused rat heart.

The labeling of PE by [1-^{14}C] arachidonic acid in the perfused rat heart was significantly affected by the thyroid condition (Table XV). Hyperthyroidism significantly decreased the labeling of cardiac PE by 37% ($P < 0.025$) and hypothyroidism decreased the labeling of cardiac PE by 57% ($P < 0.025$), compared to controls. Labeling of all other phospholipids in hearts perfused by [1-^{14}C] arachidonic acid was not significantly affected by the thyroid condition. Among the neutral lipids, labeling of TG was unaffected by the thyroid condition and labeling of DG was only significantly affected by

Table XIV

Effect of thyroid condition on incorporation of radioactivity into cardiac neutral and phospholipids in rat hearts perfused in Langendorff mode for 30 min. with Krebs Henseleit buffer containing 0.08 mM [1-^{14}C] linoleic acid (18:2). Values represent the mean ± standard deviation of at least three hearts. * P < 0.025.

Lipid	Control	Hyperthyroid dpm X 10^5 /g freeze dried ventricle	Hypothyroid
Phosphatidylethanolamine	0.73 ± 0.10	0.98 ± 0.08 *	0.63 ± 0.08
Phosphatidylcholine	5.21 ± 0.37	4.22 ± 1.12	4.86 ± 0.27
Phosphatidylserine	0.12 ± 0.01	0.10 ± 0.01	0.11 ± 0.02
Phosphatidylinositol	1.17 ± 0.30	0.81 ± 0.15	0.96 ± 0.16
Phosphatidic Acid	0.44 ± 0.05	0.24 ± 0.09	0.38 ± 0.02
Cardiolipin	0.17 ± 0.03	0.28 ± 0.03 *	0.16 ± 0.03
Phosphatidylglycerol	0.07 ± 0.02	0.06 ± 0.01	0.07 ± 0.01
Lysophosphatidylcholine	0.06 ± 0.02	0.04 ± 0.01	0.07 ± 0.04
Diacylglycerol	3.87 ± 0.60	3.06 ± 0.93	7.03 ± 2.95
Triacylglycerol	40.75 ± 5.22	14.86 ± 6.29 *	57.84 ± 6.10 *

Table XV

Effect of thyroid condition on incorporation of radioactivity into cardiac neutral and phospholipids in rat hearts perfused in Langendorff mode for 30 min. with Krebs Henseleit buffer containing 0.01 mM [1-^{14}C] arachidonic acid (20:4). Values represent the mean ± standard deviation of at least three hearts. * P < 0.025.

Lipid	Control	Hyperthyroid dpm X 10^5 /g freeze dried ventricle	Hypothyroid
Phosphatidylethanolamine	5.66 ± 0.93	3.52 ± 0.59 *	2.44 ± 0.30 *
Phosphatidylcholine	21.56 ± 4.45	27.96 ± 4.70	19.04 ± 1.93
Phosphatidylserine	0.95 ± 0.05	0.82 ± 0.16	0.94 ± 0.04
Phosphatidylinositol	3.20 ± 0.62	3.56 ± 0.74	3.55 ± 0.26
Phosphatidic Acid	0.26 ± 0.07	0.14 ± 0.06	0.24 ± 0.03
Cardiolipin	0.30 ± 0.11	0.22 ± 0.12	0.52 ± 0.21
Phosphatidylglycerol	0.09 ± 0.02	0.07 ± 0.02	0.10 ± 0.04
Lysophosphatidylcholine	0.08 ± 0.01	0.05 ± 0.01	0.10 ± 0.02
Diacylglycerol	5.83 ± 1.31	2.41 ± 0.09 *	7.68 ± 5.48
Triacylglycerol	8.95 ± 3.44	4.06 ± 2.24	13.91 ± 2.06

hyperthyroidism. In hyperthyroid animals, labeling of DG by [1-^{14}C] arachidonic acid was decreased by 59% ($P < 0.025$) compared to controls.

III. Effect of thyroid condition on phospholipid pool sizes in the heart and the microsomal and mitochondrial fractions of the heart.

To determine whether the thyroid condition affected cardiac phospholipid phosphorus mass, rat hearts were homogenized and the phospholipid extracted from the whole heart, or in other experiments from cardiac microsomal and mitochondrial fractions, and the phospholipid phosphorus determined. The total phospholipid phosphorus content of the heart was determined to be 54,420 ± 9,636 nmole/g control freeze dried heart, 65,162 ± 10,523 nmole/g hyperthyroid freeze dried heart, and 59,789 ± 5,692 nmole/g hypothyroid freeze dried heart. Therefore the total phospholipid phosphorus content of the heart was not significantly affected by the thyroid condition. As seen in Table XVI, there were no significant changes in the pool sizes of the major phospholipids in the heart. The total phospholipid phosphorus content of cardiac microsomes was determined to be 3,963 ± 93 nmole/g control wet heart, 4,007 ± 862 nmole/ g wet hyperthyroid animal heart, and 5,363 ± 1,962 nmole/g wet hypothyroid animal heart. Therefore the total phospholipid phosphorus content of rat heart microsomes was not significantly altered by the thyroid condition. In addition, the thyroid condition induced no significant changes in the pool sizes of any of the major microsomal phospholipids in the heart (Table XVII). The total phospholipid phosphorus content of cardiac mitochondria was determined to be 2,643 ± 850 nmole/g wet control heart, 2,423 ± 497 nmole/g wet hyperthyroid animal heart, and 2,810 ± 243 nmole/g wet hypothyroid animal heart. Therefore the total phospholipid phosphorus content of rat heart mitochondria was not affected by thyroid condition. Likewise, the thyroid condition had no significant effects on the phospholipid phosphorus mass of the major cardiac phospholipids in the mitochondria (Table XVIII).

Table XVI

Effect of thyroid condition on pool size of the major cardiac phospholipids. Values represent the mean ± standard deviation of at least three hearts.

Lipid	Control	Hyperthyroid nmole X 10^3/g heart	Hypothyroid
Phosphatidylethanolamine	14.8 ± 1.9	13.3 ± 0.4	17.1 ± 2.8
Phosphatidylcholine	23.8 ± 2.8	20.7 ± 5.2	27.5 ± 3.6
Cardiolipin	6.1 ± 0.1	4.6 ± 0.7	6.2 ± 0.4
Phosphatidylserine	1.3 ± 0.3	1.1 ± 0.2	1.3 ± 0.4
Phosphatidylinositol	1.1 ± 0.2	0.8 ± 0.2	1.0 ± 0.2

Table XVII

Effect of thyroid condition on pool size of the major cardiac microsomal phospholipids. Values represent the mean ± standard deviation of at least three hearts.

Lipid	Control	Hyperthyroid nmole/g heart	Hypothyroid
Phosphatidylethanolamine	416 ± 51	470 ± 26	436 ± 49
Phosphatidylcholine	617 ± 150	570 ± 89	534 ± 78

Table XVIII

Effect of thyroid condition on the pool size of the major cardiac mitochondrial phospholipids. Values represent the mean ± standard deviation of at least three hearts.

Lipid	Control	Hyperthyroid nmole/g heart	Hypothyroid
Phosphatidylethanolamine	339 ± 20	432 ± 78	412 ± 62
Phosphatidylcholine	369 ± 179	598 ± 137	412 ± 62
Cardiolipin	145 ± 49	220 ± 39	210 ± 90

IV. Effect of thyroid condition on the *de novo* biosynthetic pathways of phosphatidylethanolamine.

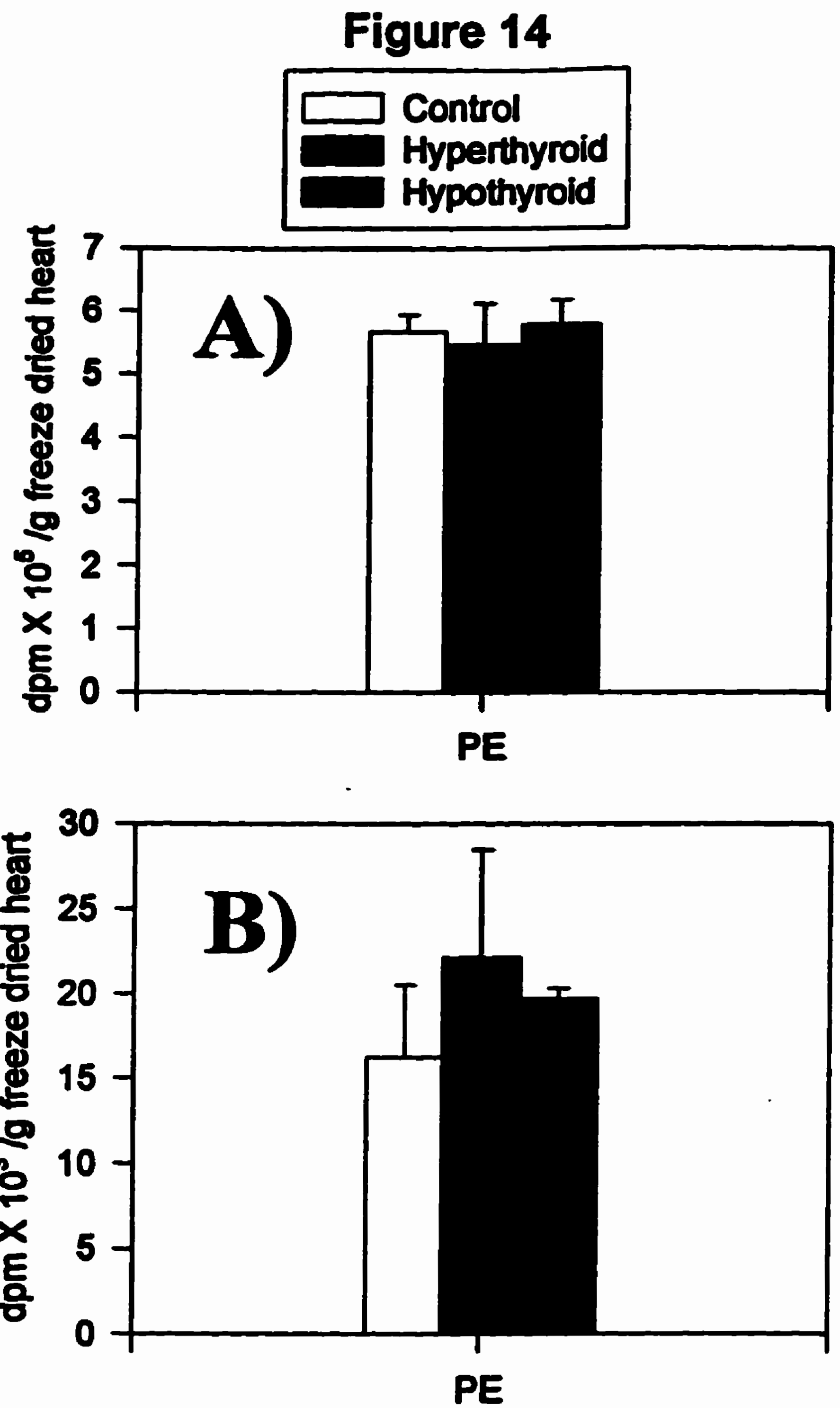
Since the thyroid condition appeared to have its most consistent effects on the incorporation of fatty acids into PE, the effect of the thyroid condition on the *de novo* biosynthetic pathways of PE was investigated.

1. CDP-ethanolamine pathway

The effect of the thyroid condition on the CDP-ethanolamine pathway was examined in rat hearts perfused in Langendorff mode for 30 min. in a continuous pulse-labeling experiment. The perfusate contained [1,2-^{14}C]ethanolamine. Subsequent to perfusion, hearts were frozen in liquid nitrogen and freeze dried. As seen in Fig. 14A, the formation of phosphatidyl[1,2-^{14}C]ethanolamine was not significantly affected by the thyroid state. In addition, the incorporation of radioactivity into the aqueous ethanolamine containing metabolites was not significantly affected by the thyroid state (Table XIX). Therefore, the thyroid condition does not affect the CDP-ethanolamine pathway.

2. Decarboxylation of phosphatidylserine

The effect of the thyroid condition on the decarboxylation of phosphatidylserine was examined in rat hearts perfused in Langendorff mode for 30 min. in a continuous pulse labeling experiment. The perfusate contained [^{3}H(G)]serine. Subsequent to perfusion, hearts were frozen in liquid nitrogen and freeze dried. As seen in Fig. 14B, the formation of phosphatidyl[^{3}H(G)]ethanolamine was not significantly affected by the thyroid state. In addition, the incorporation of radioactivity into the aqueous ethanolamine containing metabolites was not significantly affected by the thyroid state (Table XX). Therefore, the thyroid condition does not appear to affect the decarboxylation of phosphatidylserine.



Effect of thyroid condition on the *de novo* biosynthesis of phosphatidylethanolamine.

Rat hearts were perfused in Langendorff mode for 30 min. with Krebs Henseleit buff containing A) [1,2-^{14}C]ethanolamine or B) [^{3}H(G)]serine.

Vertical bars represent the mean ± standard deviation of at least three hearts.

Table XIX

Effect of thyroid condition on the *de novo* biosynthesis of phosphatidylethanolamine. Rat hearts were perfused in Langendorff mode for 30 min. with Krebs Henseleit buffer containing [1,2-^{14}C] ethanolamine and the radioactivity incorporated into aqueous ethanolamine containing compounds measured. Values represent the mean ± standard deviation of at least three hearts.

Thyroid Condition	Phosphoethanolamine	CDP-ethanolamine	Ethanolamine
	dpm X 10^3/ g freeze dried ventricle		
Control	47.0 ± 6.0	1.9 ± 0.4	31.4 ± 9.5
Hyperthyroid	47.0 ± 8.0	1.0 ± 0.2	41.7 ± 2.1
Hypothyroid	68.8 ± 11.1	1.4 ± 0.5	31.0 ± 5.2

Table XX

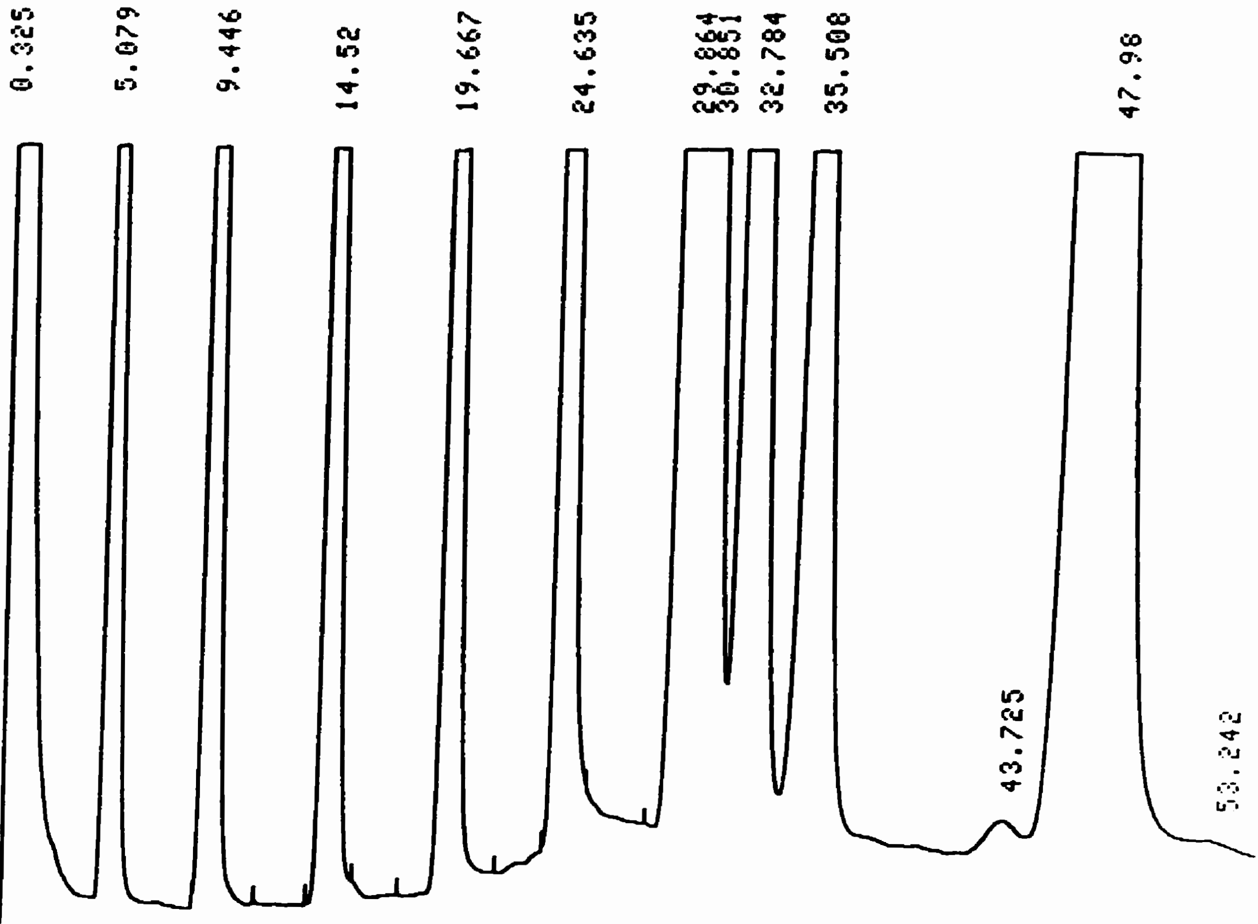
Effect of thyroid condition on the *de novo* biosynthesis of phosphatidylethanolamine. Rat hearts were perfused in Langendorff mode for 30 min. with Krebs Henseleit buffer containing [^{3}H(G)] serine and the radioactivity incorporated into aqueous ethanolamine containing compounds measured. Values represent the mean ± standard deviation of at least three hearts.

Thyroid Condition	Phosphoethanolamine	CDP-ethanolamine	Ethanolamine
	dpm X 10^3/ g freeze dried ventricle		
Control	15.7 ± 5.8	1.2 ± 0.4	27.5 ± 6.3
Hyperthyroid	16.1 ± 6.8	0.8 ± 0.3	28.0 ± 5.1
Hypothyroid	9.2 ± 5.0	1.4 ± 0.4	44.9 ± 9.8

V. Effect of thyroid condition on the fatty acid molecular composition of cardiac neutral and phospholipids.

Since the thyroid condition induced changes in the incorporation of [1-^{14}C] fatty acids into neutral and phospholipids in the perfused rat heart, the effect of thyroid condition on the fatty acid molecular composition of both neutral and phospholipids in the heart was investigated. Methyl esters of fatty acids from isolated cardiac neutral and phospholipids were prepared. These methyl esters were separated using a Shimadzu GC-14A gas chromatograph. The average retention time of this particular system for palmitic acid (16:0) was 24.6 min., stearic acid (18:0) 29.9 min., oleic acid (18:1) 30.9 min., linoleic acid (18:2) 32.8 min., and arachidonic acid (20:4) 48.0 min. (Fig. 15). The peak areas generated were used to quantify the percentage of the total fatty acid. It was found that in hyperthyroid animals, there was a significant 47% increase ($P < 0.025$) in oleic acid (18:1) content of cardiac PE, compared to controls (Table XXI). Changes in the other major fatty acids of PE were not significant. In hypothyroid rats, there was a significant 1.3 fold increase ($P < 0.025$) in the palmitic acid (16:0) content of PE compared to controls. There were no significant changes in the other major fatty acids of cardiac PE from hypothyroid animals compared to controls. Interestingly, the thyroid state did not significantly affect the fatty acid molecular composition of PC (Table XXII), PI (Table XXIII), PS (Table XXIV), PG (Table XXV), nor DG (Table XXVII). Hyperthyroidism significantly increased the oleic acid content of CL by 1.5 fold ($P < 0.025$) compared to controls without significantly changing the composition of its other fatty acids (Table XXVI). Hyperthyroidism also significantly increased the stearic acid (18:0) content of TG by 1.2 fold ($P < 0.025$) compared to controls without significantly changing the content of its other fatty acids (Table XXVIII).

Figure 15



Gas Chromatographic Separation of Methylester Standards

Fatty Acid	*Retention Time* (min)
Heptane Solvent	0.325
8:0	5.079
10:0	9.446
12:0	14.52
14:0	19.667
16:0	24.635
18:0	29.864
18:1	30.851
18:2	32.784
18:3	35.508
20:4	47.98

Table XXI

Effect of thyroid condition on fatty acid composition of cardiac phosphatidylethanolamine. Values represent the mean ± standard deviation of at least three hearts. * $P < 0.025$.

Fatty Acid		Percent Fatty Acid	
	* Control	Hyperthyroid	Hypothyroid
16:0	8.0 ± 0.4	8.9 ± 0.6	10.5 ± 1.1 *
18:0	29.9 ± 2.2	30.4 ± 0.8	32.7 ± 4.4
18:1	4.5 ± 0.1	6.6 ± 0.4 *	5.1 ± 1.4
18:2	7.5 ± 0.1	8.3 ± 0.4	6.0 ± 0.4
20:4	48.5 ± 1.5	44.5 ± 2.7	43.8 ± 2.2
others	1.6 ± 0.2	1.3 ± 0.5	1.9 ± 0.3

Table XXII

Effect of thyroid condition on fatty acid composition of cardiac phosphatidylcholine. Values represent the mean ± standard deviation of at least three hearts.

Fatty Acid	Percent Fatty Acid		
	Control	Hyperthyroid	Hypothyroid
16:0	20.7 ± 2.7	19.4 ± 0.2	20.4 ± 3.0
18:0	29.2 ± 4.0	28.3 ± 0.6	26.8 ± 2.4
18:1	10.1 ± 1.3	9.9 ± 0.2	8.0 ± 0.7
18:2	12.6 ± 3.1	15.2 ± 1.1	8.9 ± 2.6
20:4	30.3 ± 2.8	26.8 ± 2.5	35.8 ± 4.2
others	0.0 ± 0.0	0.4 ± 0.1	0.5 ± 0.4

Table XXIII

Effect of thyroid condition on fatty acid composition of cardiac phosphatidylinositol. Values represent the mean ± standard deviation of at least three hearts.

Fatty Acid	Percent Fatty Acid		
	Control	Hyperthyroid	Hypothyroid
16:0	6.5 ± 1.2	7.8 ± 2.7	9.11 ± 1.3
18:0	47.4 ± 1.7	53.6 ± 4.0	41.2 ± 5.1
18:1	9.7 ± 2.1	13.4 ± 4.0	10.7 ± 4.0
18:2	5.0 ± 1.2	5.9 ± 0.4	5.0 ± 0.7
20:4	25.9 ± 2.4	21.5 ± 5.1	27.6 ± 1.7
others	2.4 ± 0.9	2.3 ± 0.6	4.3 ± 0.4

Table XXIV

Effect of thyroid condition on fatty acid composition of cardiac phosphatidylserine. Values represent the mean ± standard deviation of at least three hearts.

Fatty Acid	Percent Fatty Acid		
	Control	Hyperthyroid	Hypothyroid
16:0	10.8 ± 3.0	16.0 ± 2.4	10.5 ± 2.4
18:0	59.1 ± 5.2	54.4 ± 3.5	56.5 ± 5.9
18:1	12.9 ± 4.5	16.9 ± 3.1	9.4 ± 0.5
18:2	3.1 ± 0.8	3.8 ± 0.4	2.9 ± 0.3
20:4	15.6 ± 3.3	9.5 ± 0.9	19.2 ± 8.2
others	3.5 ± 1.2	6.4 ± 2.2	1.6 ± 0.4

Table XXV

Effect of thyroid condition on fatty acid composition of cardiac phosphatidylglycerol. Values represent the mean ± standard deviation of at least three hearts.

Fatty Acid	Percent Fatty Acid		
	Control	Hyperthyroid	Hypothyroid
16:0	36.8 ± 1.0	36.1 ± 1.6	34.1 ± 0.8
18:0	15.5 ± 2.2	14.3 ± 1.4	20.8 ± 2.6
18:1	35.9 ± 3.9	39.0 ± 4.7	35.8 ± 5.7
18:2	10.1 ± 0.4	8.3 ± 1.7	6.7 ± 0.7
20:4	1.6 ± 1.5	1.8 ± 1.9	2.6 ± 4.4
others	N.D.	0.5 ± 0.5	N.D.

Table XXVI

Effect of thyroid condition on fatty acid composition of cardiac cardiolipin. Values represent the mean ± standard deviation of at least three hearts. * P < 0.025.

Fatty Acid	Percent Fatty Acid		
	Control	Hyperthyroid	Hypothyroid
16:0	1.5 ± 0.1	2.8 ± 0.7	2.5 ± 1.6
18:0	1.6 ± 0.3	3.8 ± 1.7	2.9 ± 1.8
18:1	7.7 ± 1.8	11.4 ± 0.6 *	12.7 ± 1.3
18:2	87.2 ± 2.4	78.7 ± 5.1	82.5 ± 8.6
20:4	2.0 ± 0.1	3.4 ± 2.6	3.5 ± 3.1
others	N.D.	N.D.	N.D.

Table XXVII

Effect of thyroid condition on fatty acid composition of cardiac diacylglycerol. Values represent the mean ± standard deviation of at least three hearts.

Fatty Acid	Percent Fatty Acid		
	Control	Hyperthyroid	Hypothyroid
16:0	21.3 ± 3.0	22.6 ± 2.4	20.6 ± 1.9
18:0	22.0 ± 4.0	24.7 ± 4.2	17.4 ± 5.4
18:1	30.6 ± 4.5	35.2 ± 1.5	33.6 ± 0.3
18:2	10.4 ± 2.2	6.4 ± 5.6	6.7 ± 1.6
20:4	3.9 ± 3.6	N.D.	N.D.
others	11.7 ± 1.4	11.1 ± 4.5	11.8 ± 0.7

Table XXVIII

Effect of thyroid condition on fatty acid composition of cardiac triacylglycerol. Values represent the mean ± standard deviation of at least three hearts. * P < 0.025.

Fatty Acid	Percent Fatty Acid		
	Control	Hyperthyroid	Hypothyroid
16:0	22.5 ± 3.4	19.8 ± 2.9	26.6 ± 0.5
18:0	8.5 ± 0.6	10.8 ± 0.5 *	18.0 ± 0.4 *
18:1	35.1 ± 1.5	39.8 ± 1.7	31.4 ± 4.3
18:2	19.6 ± 6.7	16.7 ± 0.5	14.4 ± 3.4
20:4	7.1 ± 2.8	3.4 ± 1.5	N.D.
others	7.1 ± 2.1	9.6 ± 0.8	9.7 ± 1.9

VI. Effect of thyroid condition on the *in vitro* enzyme activities involved in phospholipid remodeling.

1. Effect of thyroid state on long chain fatty acyl-CoA synthetase activity.

To examine whether the thyroid condition induced changes in the oleic acid content of PE in the heart were due to the increased availability of long chain fatty acyl-CoAs, the activity of long chain fatty acyl-CoA synthetase was measured in heart microsomes and mitochondria. Using [1-^{14}C]oleic acid as the substrate, the activity of long chain fatty acyl-CoA synthetase was discovered to be unaltered by the thyroid state (Table XXIX).

2. Effect of thyroid state on acyltransferase activities.

Since the most dramatic effects of thyroid state appeared to be directed towards fatty acid metabolism involving PE, the effect of the thyroid state on LPE AT was investigated using [1-^{14}C] oleoyl-CoA as the substrate. The activity of LPE AT was increased by 1.74 fold ($P < 0.025$) in cardiac microsomal fractions prepared from hyperthyroid animals, compared with controls (Table XXX). The activity of cardiac microsomal LPE AT was decreased by 23% ($P < 0.025$) in hypothyroid animals compared with controls. The activities of mitochondrial LPE AT activity was unaltered by the thyroid state of the animal. Cytosolic LPE AT activity was also unaltered by the thyroid state, however this activity was likely due to contamination by microsomal particles (Hatch and Choy, 1987). In addition, the activity of microsomal LPC AT in the heart was measured and discovered to be unaffected by the thyroid state of the rat (Table XXXI).

3. Effect of thyroid state on the activity of cardiac phospholipase A.

The effect of the thyroid condition on cardiac phospholipase A activity was examined in rat heart microsomes, mitochondria and cytosol. When the activity towards phosphatidyl[1,2-^{14}C]ethanolamine was measured, the activities of microsomal and

Table XXIX

Effect of thyroid condition on the activity of long chain fatty acyl-CoA synthetase in rat heart sub-cellular fractions. Values represent the mean ± standard deviation of at least three hearts.

	Specific activity of long chain fatty acyl-CoA synthetase nmole/min per mg protein	
	Microsomes	Mitochondria
Control	61.25 ± 1.37	40.27 ± 5.04
Hyperthyroid	62.02 ± 4.80	32.81 ± 5.37
Hypothyroid	54.36 ± 2.37	39.81 ± 0.38

Table XXX

Effect of thyroid condition on the activity of acyl-CoA:1-acylglycerophosphoethanolamine acyltransferase in rat heart sub-cellular fractions. Values represent the mean ± standard deviation of at least three hearts. * P < 0.025.

Thyroid Condition	Specific activity of acyl-CoA:1-acylglycerophosphoethanolamine acyltransferase nmole/min per mg protein Microsomes	Mitochondria	Cytosol
Control	0.296 ± 0.009	0.088 ± 0.032	0.027 ± 0.005
Hyperthyroid	0.514 ± 0.086 *	0.085 ± 0.011	0.022 ± 0.002
Hypothyroid	0.228 ± 0.025 *	0.076 ± 0.006	0.029 ± 0.006

Table XXXI

Effect of thyroid condition on the activity of acyl-CoA:1-acylglycerophosphocholine acyltransferase in rat heart microsomes. Values represent the mean ± standard deviation of at least three hearts.

Thyroid Condition	Specific activity of acyl-CoA:1-acylglycerophosphocholine acyltransferase nmoles/min per mg protein Microsomes
Control	33.65 ± 0.41
Hyperthyroid	30.88 ± 2.03
Hypothyroid	32.47 ± 2.55

cytosolic phospholipase A were not significantly affected by the thyroid condition (Table XXXII). However, the mitochondrial phospholipase A activity was significantly decreased by 20% ($P < 0.025$) in hyperthyroid animals and by 50% ($P < 0.025$) in hypothyroid animals, compared with controls.

In addition, the activity of cardiac phospholipase A towards [1,2-^{14}C-dipalmitoyl]phosphatidylcholine was measured in rat heart microsomes, mitochondria and cytosol. The activity of microsomal and cytosolic phospholipase A was decreased by 30% and 58% ($P < 0.025$) respectively, in hyperthyroid animals compared with controls (Table XXXIII). However, hypothyroidism did not significantly affect the activity of phospholipase A in heart microsomes and cytosol, compared to controls. Mitochondrial phospholipase A activity towards [1,2-^{14}C-dipalmitoyl]phosphatidylcholine was unaffected by the thyroid state of the animal.

Table XXXII

Effect of thyroid condition on the activity of phospholipase A towards phosphatidyl[1,2-^{14}C]ethanolamine in rat heart sub-cellular fractions. Values represent the mean ± standard deviation of at least three hearts. * P < 0.025.

	Specific activity of phospholipase A nmole/min per mg protein		
	Microsomes	Mitochondria	Cytosol
Control	0.390 ± 0.024	0.314 ± 0.014	0.181 ± 0.035
Hyperthyroid	0.414 ± 0.029	0.246 ± 0.006 *	0.241 ± 0.038
Hypothyroid	0.438 ± 0.096	0.154 ± 0.009 *	0.383 ± 0.102

Table XXXIII

Effect of thyroid condition on the activity of phospholipase A towards [1,2-^{14}C-dipalmitoyl]phosphatidylcholine in rat heart sub-cellular fractions. Values represent the mean ± standard deviation of at least three hearts. * P < 0.025.

Thyroid Condition	Specific activity of phospholipase A nmole/min per mg protein		
	Microsomes	Mitochondria	Cytosol
Control	0.151 ± 0.021	0.120 ± 0.020	0.416 ± 0.064
Hyperthyroid	0.106 ± 0.007 *	0.107 ± 0.016	0.175 ± 0.026 *
Hypothyroid	0.136 ± 0.035	0.095 ± 0.015	0.381 ± 0.125

DISCUSSION

The objective of this study was to determine whether the thyroid condition of the animal influenced the remodeling of cardiac phospholipids. The effect of thyroid hormones on the alteration of the membrane lipid composition is well established (Hoch, 1988). Previously, it has been shown that T_3 treatment significantly decreased the activity of rat liver microsomal LPC AT by 28% compared to controls (Dang *et al.*, 1985). Hypothyroidism increased rat liver microsomal LPC AT activity by 14% and this may contribute to increased proportions of unsaturated fatty acids found in the phospholipids from the livers of hypothyroid rats (Dang *et al.*, 1985). Therefore changes in the activities of acyltransferase enzymes and their regulation are important for establishing the fatty acid composition of a particular phospholipid.

The results presented in this study clearly demonstrated that the incorporation of oleic acid into cardiac PE was elevated in hyperthyroidism. We have observed that the phospholipid phosphorous mass of PE and *de novo* biosynthesis of PE in the heart was not significantly affected by the thyroid condition of the rat (Table XVI). This was in agreement with previous findings in the heart that showed that the phosphorus mass of PE was unaffected by the thyroid condition of the rat (Paradies *et al.*, 1993; 1994). However, the fatty acid composition of cardiac PE showed a significant 47% increase in its oleic acid content (Table XXI). In addition, there was a significant 50% increase in the incorporation of [1-^{14}C]oleic acid into PE in the isolated perfused rat heart (Table XIII). These agree with findings in the livers of hyperthyroid rats that showed a 68% increase in the oleic acid content of PE in livers from hyperthyroid rats (Raederstorff *et al.*, 1991). The remodeling of PE is an important process since only a limited number of its molecular species were synthesized through the *de novo* pathway (Schmid *et al.*, 1995). The mechanism for the increased oleic acid content of PE was likely a consequence of the 74%

elevation in the activity of cardiac microsomal LPE AT (Table XXX). The fatty acid composition of cardiac PE from hypothyroid rats showed a significant 30% increase in its palmitic acid content (Table XXI). In addition, there was also a 4-fold increase in the incorporation of [1-^{14}C]palmitic acid into PE in the isolated perfused rat heart (Table XII). Previously, only a 14% increase in the PE palmitic acid content was observed in the livers of hypothyroid rats (Raederstorff *et al.*, 1991). Since most of the palmitic acid content of PE arises from the *de novo* biosynthetic pathway (Holub and Kuksis, 1978; Schmid *et al.*, 1995). The increased palmitic acid content of PE was likely a consequence of the 23% decreased activity of LPE AT observed in microsomes from the hearts of hypothyroid rats (Table XXX). Even though it has been suggested that the intracellular acyl-CoA concentration plays an important role in determining the fatty acid composition of PE (Arthur *et al.*, 1987a), we have shown that the activity of fatty acyl-CoA synthetase was unaffected by the thyroid condition (Table XXIX). This indicated that the acyl-CoA concentration might not be changed by the thyroid condition. The activity of cardiac microsomal and cytosolic phospholipase A towards PE was unaffected by the thyroid condition (Table XXXI) suggesting that the concentration of LPE in the heart was not elevated due to the thyroid condition.

The finding that hyperthyroidism elevated LPE AT activity, while hypothyroidism decreased LPE AT activity is the first example showing that PE remodeling may be regulated by thyroid hormones in the heart. The significance of this finding may have implications towards understanding the growth and differentiation of mammalian cardiac tissue, since the regulation of these events remain poorly understood. Embryonic P19 tetratocarcinoma cells have the ability to differentiate into either neurons or cardiac and skeletal muscle cells when provided with the appropriate stimulus (McBurney *et al.*, 1982). It has been shown that cardiac differentiation of P19 cells could be induced by T_3

treatment of cells and differentiation to neurons or skeletal muscle cells was absent (Rodriguez *et al.*, 1994). In Table VII, it was observed that the thyroid condition dramatically affects the heart ventricular weight. Since LPE AT activity increased with the increasing heart weight observed in hyperthyroid rats and decreased with the decreasing heart weight observed in hypothyroid rats, the hormonal control of LPE AT activity must be important in the development of cardiac tissue. Clejan *et al.* (1980) originally suggested that the primary action of T_4 at the membrane is on membrane fluidity and other metabolic effects are secondary. Since PE is a major membrane phospholipid, alteration of its fatty acid composition could affect the responsiveness of membrane bound receptors as well as facilitate more rapid addition of membrane lipid through modifications of its fatty acid composition to allow the membrane to be more fluid. Perhaps, thyroid hormones act as signals that eventually lead to changes in the content of oleic over palmitic acid. Increasing the content of unsaturated fatty acids would increase membrane fluidity, thereby permitting more rapid growth of cardiac membranes, whereas increasing the content of saturated fatty acids would increase membrane rigidity, thereby slowing growth of cardiac membranes. At the same time alterations of membrane fluidity would influence the activity of various membrane associated enzymes and receptors. Since the induction of differentiation of P19 cells by T_3 is accompanied by expression of cardiac specific genes (Rodriguez *et al.*, 1994), the LPE AT gene may be one of the targets for thyroid hormones.

SUMMARY

In this study, the effect of the thyroid condition of the animal on phospholipid remodeling was studied in the rat heart. Perfusion of the heart and gas chromatographic analysis revealed that there was an increased incorporation of oleic acid into cardiac PE from hyperthyroid animals compared to controls. The mechanism for the increased oleic acid incorporation was likely the 1.74 fold increase in the activity of microsomal LPE AT observed in hyperthyroid rats. Perfusion of the heart and gas chromatographic analysis showed that there was an increased incorporation of palmitic acid into cardiac PE from hypothyroid animals compared to controls. The mechanism for the increased palmitic acid incorporation was likely the 23% decrease in the activity of microsomal LPE AT observed in hypothyroid rats. These results provide the first evidence that the remodeling of cardiac PE is controlled by thyroid hormones.

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