TRIACYLGLYCEROL SYNTHESIS IN RAT ADIPOSE TISSUE

A thesis

presented in partial fulfilment of the

requirements for the degree of

Master of Science in Biochemistry

at Massey University

by

Russell Douglas Wilson

December, 1983

ACKNOWLEDGEMENTS

The author wishes to thank Dr J.C. Hawke (Chemistry, Biochemistry and Biophysics Department, Massey University) for his excellent supervision and advice throughout the course of this study.

The author also expresses his appreciation to the following persons for their assistance:

Mr J.C.W. Reid for assistance with experimental animals.

Mrs Glenda Shaw for typing this thesis.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

AMP - adenosine 5'-monophoshate

ATP - adenosine 5'-triphoshate

B.P. - boiling point

B.S.A. - bovine serum albumin

cAMP - adenosine 3',5'-monophosphate

Ci - curie

CoA - coenzyme A

DG - diacylglycerol

DGAT - diacylglycerol acyltransferase

DHAP - dihydroxyacetone phosphate

DHAPAT - dihydroxyacetone phosphate acyltransferase

EDTA - ethylenediaminetetraacetic acid

E.R. - endoplasmic reticulum

FFA - free fatty acid

G-3-P - sn-glycerol-3-phosphate

GPAT - sn-glycerol-3-phosphate acyltransferase

I.U. - international unit

MAGAT - monoacylglycerol acyltransferase

MAGPAT - monoacylglycerol phosphate acyltransferase

MES - 2-[N-morpholino]ethanesulfonic acid

MG - monoacylglycerol

PA - phosphatidic acid

PAP - phosphatidic acid phosphatase

PC - phosphatidylcholine

PE - phosphatidylethanolamine

PL - phospholipid

POPOP - 1,4 bis(2-(5-phenyloxazolyl))-benzene

PPO - 2,5-diphenyl oxazole

S.D. - standard deviation

sn - stereospecific numbering

TG - triacylglycerol

Tris - tri-(hydroxymethyl)methylamine

Lipid nomenclature

For the specific structural designation of complex lipids containing a glycerol moiety, the nomenclature suggested by the IUPAC-IUB Commision on Biochemical Nomenclature (European J. Biochem.(1967) 2,127). The trivial names of complex lipids have been used when it is more appropiate to do so.

Fatty acids are designated by the shorthand notation of the number of carbons atoms: number of double bonds, e.g. 16:0 refers to hexadecanoic acid (palmitic acid).

CHAPTER 1

Review of literature

1.1 Introduction

Adipose tissue consists of two types of cell; the adipocyte and the non-adipocyte (Rodbell, 1964). The adipocyte is unique amongst mammalian cell types in that one class of component (TG) comprises up to 80% of its weight. These TG's constitute the major energy storage material in higher animals. The importance of adipose tissue in mammalian metabolism lies in its ability to store free-fatty acids (FFA) as TG and to release them again according to physiological demands. It is therefore understandable that the pathways of synthesis and degradation should be under strict control. Apart from adipose tissue TG metabolism is especially active in the mammary gland during lactation and in the liver and intestinal mucosa cells during synthesis and secretion of serum lipoproteins.

The general pathway of TG synthesis in adipose tissue as proposed by Kennedy and co-workers (1961), according to Figure 1-1, involves the step wise acylation of sn-glycerol-3-phosphate (G-3-P) by acyl-CoA thioesters. This G-3-P pathway is accepted as being quantitatively the most important, but acylation of dihydroxyacetone phoshate (DHAP) (Hajra, 1972) and monoacylglycerol (MG) are also known to occur. As well as the synthesis of TG the acylation of G-3-P and subsequent dephosphorylation to diacylglycerol (DG) are steps involved in the synthesis of the glycero-phospholipids (PL), phosphatidylcholine (PC) and phosphatidylethanolamine (PE) (Figure 1-2). Significant progress has been made in understanding the hormonal control of TG degradation via the cyclic 3',5' AMP-dependant activation of hormone sensitive

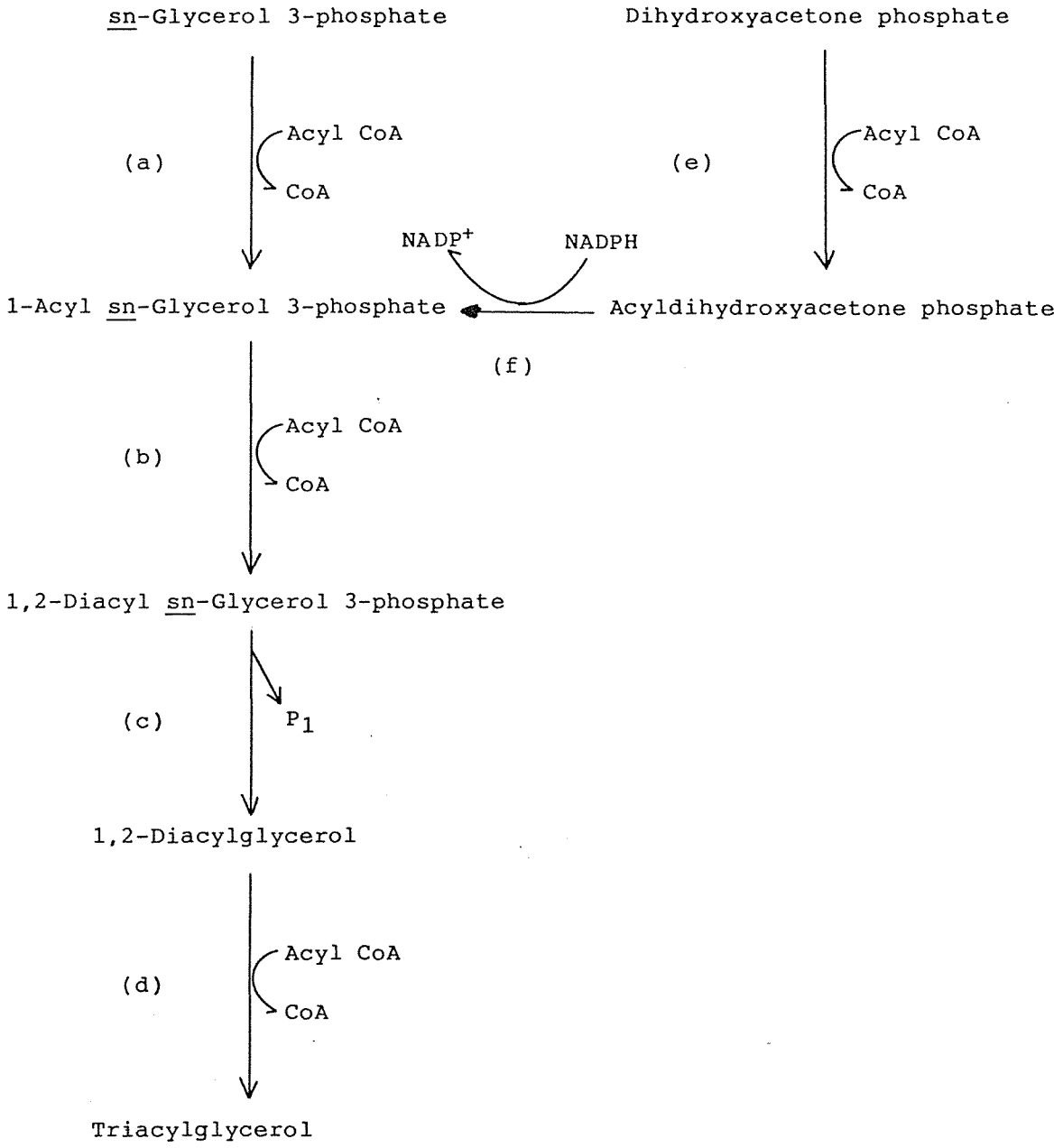


Figure 1-1. Pathways of triacylglycerol synthesis in adipose tissue. The enzymes involved are (a) sn-glycerol 3-phosphate 1-acyltransferase (GPAT), (b) 1-acylglycerol 3-phosphate acyltransferase, (c) phosphatidate phosphatase (d) diacylglycerol acyltransferase, (e) dihydroxyacetone phosphate acyltransferase (f) 1-acylglycerol 3-phosphate dehydrogenase.

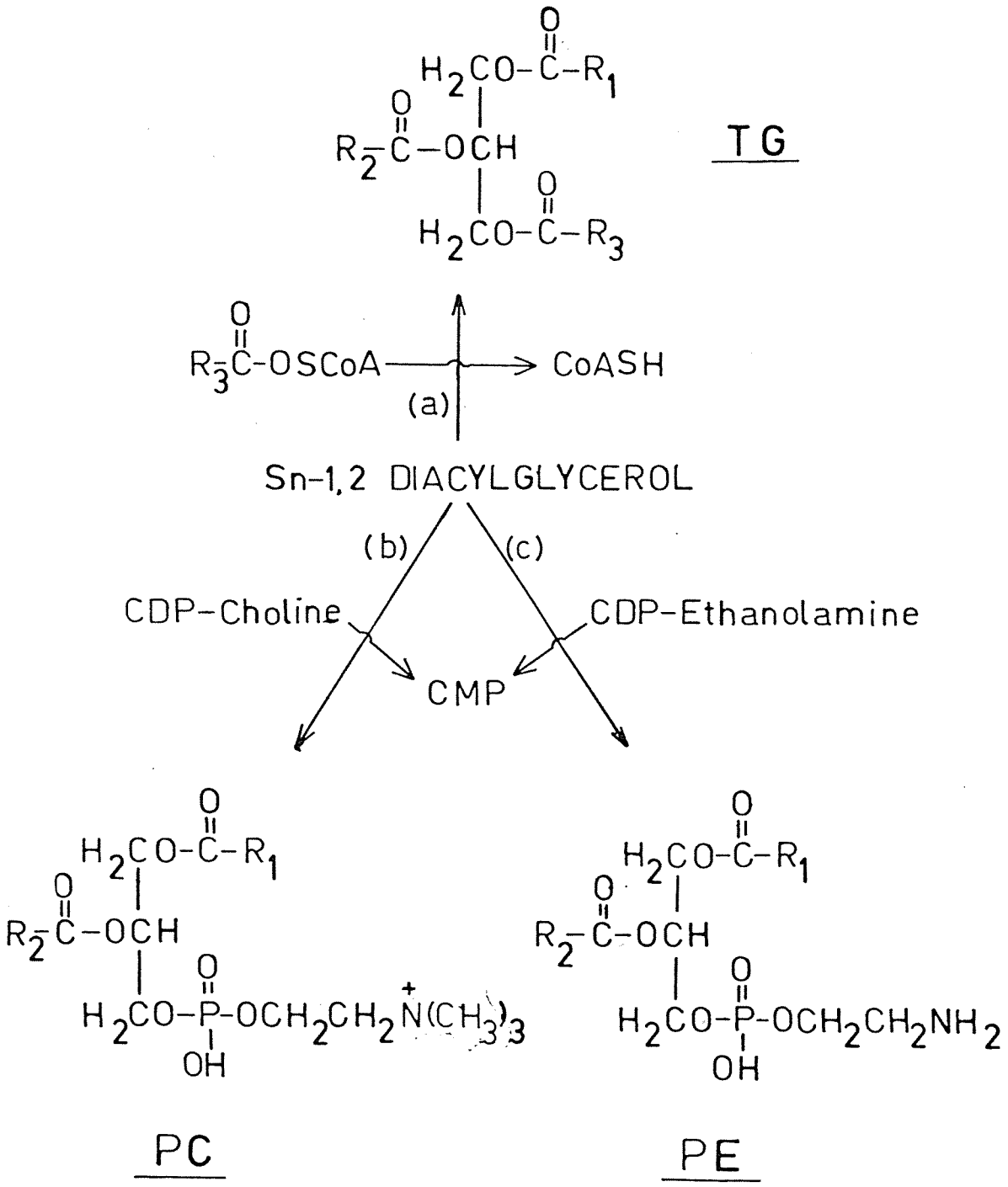


Figure 1-2. Synthesis of triacylglycerol, phosphatidylcholine and phosphatidylethanolamine from sn-1,2 diacylglycerol.

The enzymes involved are (a) diacylglycerol acyltransferase, (b) cholinephosphotransferase, (c) ethanolaminephosphotransferase. TG : triacylglycerol, PC : phosphatidylcholine, PE : phosphatidylethanolamine, CDP : cytidine 5'-diphosphate, CMP : cytidine 5'-monophosphate.

lipase (Khoo, et al. 1974). However, knowledge concerning the mechanisms regulating the synthesis of TG remain incomplete. The major question is how glycerolipid metabolism is regulated to give rise to an appropriate mixture of products to meet the demands of energy storage and utilisation, membrane biogenesis and lipoprotein biosynthesis.

1.2 Cellular location of triacylglycerol synthesis

It has been shown that the principle site of TG and PL synthesis occurs at the endoplasmic reticulum (ER). The products of these pathways are either incorporated into the adipocyte membranes (PL's) or transferred to the storage lipids (TG's). Electron microscope studies of adipocytes have shown that the smooth ER cisternae are applied to the surface of the fat droplet (Cited by Giacobino, 1979). Localisation of the enzymes at the ER has been facilitated by studies showing that microsomal vesicles maintain the orientation of the ER (DePierre and Dallmer, 1975). Coleman and Bell (1978) found that treatment of rat liver microsomes with chymotrypsin or pronase lead to the inactivation of acyl-CoA synthetase, dihydroxyacetone phosphate acyltransferase (DHAPAT), monoacylglycerolphosphate acyltransferase (MAGPAT) and diacylglycerol acyltransferase (DGAT). This would indicate that the critical regions of TG synthesis are exposed on the cytoplasmic surface. This coupled with the inability of ATP and palmitoyl-CoA (16:0 CoA) to penetrate microsomal vesicles would tend to indicate that the active sites are at the cytoplasmic surface. This orientation would allow ready access to FFA's, CoA and ATP which are required for fatty acid activation, and access to CDP-choline and CDP-ethanolamine (synthesised by soluble cytoplasmic enzymes) for PL synthesis.

1.3 Fatty acid activation

The activation of FFA's to acyl-CoA thioesters by one of several chain-length dependent acyl-CoA synthetases (Groote, et al. 1976) is required prior to acylation. TG's contain predominantly long chain FA's (Van Golde et al. 1974) involving activation by long-chain acyl-CoA synthetase (EC 6.2.1.3)

$$R\text{-}\overset{O}{\overset{\|}{C}}\text{-}OH + CoASH + ATP \longrightarrow R\text{-}\overset{O}{\overset{\|}{C}}\text{-}SCoA + AMP + PPi$$ (Khoo and Steinberg, 1974)

Detailed knowledge of acyl-CoA synthetase from work with bacterial systems is available (Coleman and Bell, Review 1980) but little information exists concerning eucaryote systems. Long-chain acyl-CoA synthetase has been solubilized from chicken adipose tissue to reveal a protein of 90,000 daltons (Banis and Tove, 1974). Jason et al. (1976) have characterised this activity using the microsomal fraction from isolated fat cells. They established that 80% of activity was associated with the microsomal fraction, they also established the kinetics for the various FA's. A two-fold increase in activity was demonstrated when isolated fat cells were incubated with physiological levels of insulin. The increase was both rapid and consistent regardless of using radioactively labelled CoA or FFA. This effect of insulin on acyl-CoA synthetase would serve to promote the capture of FFA's and hence promote TG synthesis. Also the rapid response would serve to control the distribution of catabolic fuel as requirements change. In conjunction Sooranna and Saggerson (1978c) have reported a 30-40% decrease in acyl-CoA synthetase activity in homogenates prepared from freeze-stopped adipocytes incubated with adrenalin. The effect however was relatively slow (30 mins) compared to the more rapid effects of insulin (2 mins). The effects of adrenalin could be blocked by the β blocker propanolol and by insulin. Insulin alone, however, had no

effect. It could be that the effects of adrenalin are secondary to a increase in the rate of lipolysis. The lack of stimulation by insulin is in contrast to Jason et al. (1976) but it should be remembered that different assay systems were employed.

The critical micelle concentration of acyl-CoA thioesters is 3-4 μM (Zahler, et al. 1968). Acyl-CoA's when added to incubations are usually added in excess of this concentration and it has been observed that the incorporation of 16:0 in the presence of CoA and ATP is greater than that of 16:0-CoA. Adipose tissue, as in the case of brain (De Jiminez and Cleland, 1969) and liver (Abou-Issa and Cleland, 1969), does not contain a pool of endogenous acyl-CoA thioesters.

1.4 Substrates for esterification of acyl-CoA thioesters

There are three initial acylations of three different substrates that can lead to TG synthesis in adipose tissue. They are the acylation of G-3-P, DHAP and MG. There has been some conjecture as to whether the acylation of G-3-P and DHAP are catalysed by a single enzyme. The distribution of both acylation activities in adipose tissue have been shown to be essentially the same (Schlossman and Bell, 1976). It has also been demonstrated that the acylation of each substrate is competitively inhibited by the other substrate while both acylation activities respond identically to N-ethylmaleimide, trypsin and detergent treatment (Schlossman and Bell, 1976). The failure of G-3-P to inhibit DHAPAT completely is probably due to the dual localisation of the enzyme in adipose tissue with the G-3-P-insensitive activity located in the mitochondria (Dodds, et al. 1976).

With rat liver slices it was found that 50-60% of the glycerol

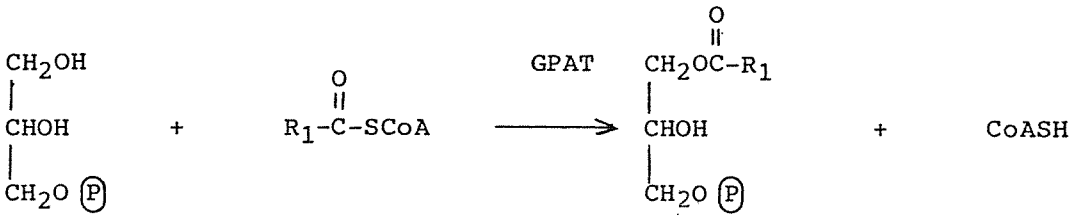
incorporated into lipid proceeded by the DHAP pathway (Manning and Brindley, 1972). In rat adipocytes the levels of G-3-P and DHAP have been estimated to be approximately 8.1 and 2.9 nmoles g^{-1} respectively (Ballard, 1972). Using these concentrations and known kinetic parameters it has been calculated that the ratio of G-3-P to DHAP acylation is greater than 12.0. The existence of a single enzyme would simplify the regulation of these committed steps of TG/PL synthesis, while the existence of two separate enzymes would complicate regulation unless they were coordinately controlled.

The role to the enzyme involved in the third acylation, MAGAT, is relatively unknown. This enzyme may play a role in the reesterification of MG's which are absorbed from the blood or it may be involved in the recycling of MG (derived from lipolysis) back into TG.

1.5 Enzymes of triacylglycerol synthesis

1.5.1 Glycerol-3-phosphate acyltransferase (GPAT)

The first step in the glycerophosphate pathway of TG synthesis is the acylation of G-3-P which is catalysed by the enzyme GPAT (EC 2.3.1.15).



G-3-P lyso-phosphatidic acid

In adipose tissue GPAT is almost exclusively located in the microsomal fraction (Schlossman and Bell, 1976) in contrast to the liver where activity is found more equally in the microsomal and mitochondrial fractions. Due to the differing responses to the thiol group reagents

iodoacetamide and N-ethylmaleimide (Bates and saggerson, 1977) it has been proposed that the two hepatic activities are due to isoenzymes of GPAT (Nimmo, 1979).

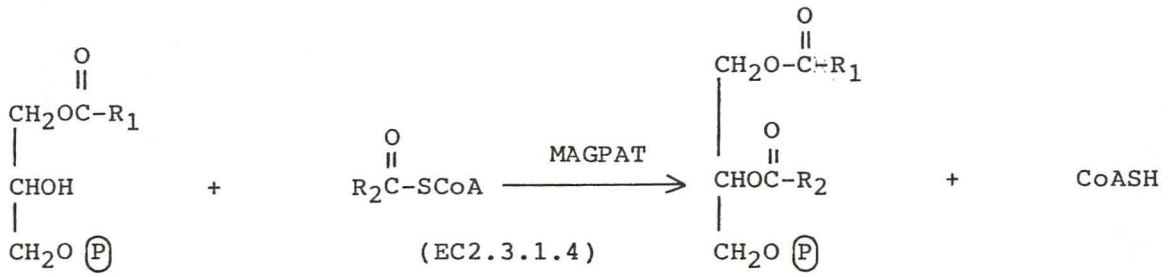
In assays where a whole tissue homogenate is used the product of G-3-P acylation is dependant on the assay conditions. Lysophosphatidic acid is not usually an isolatable intermediate of the synthetic pathway with acylation proceeding through to phosphatidic acid (PA). Increasing the amount of albumin in the medium favours formation of lysophosphatidic acid whereas longer incubations and increased amounts of microsomal protein tends to favour PA as the isolatable intermediate (Zaror-Behrens and Kako, 1976). The initial acylation of G-3-P is exclusively in the sn-1 position with little substrate specificity. This is in contrast to hepatic GPAT which preferentially utilises 16:0 CoA compared to 18:1 CoA and 18:2 CoA (Haldar and Pullman, 1975).

Being the first reaction of the TG/PL biosynthetic pathways GPAT has received considerable attention as a possible control site of the pathway. Incubation of rat adipocytes with adrenalin results in a decrease in GPAT activity (Sooranna and Saggerson, 1978b). Although there is an accompanying accumulation of FFA's it seems unlikely that the decrease in GPAT activity is due entirely to this, as there was no further decrease in GPAT activity when 16:0 was added in a concentration in excess of 3.5 mM (Sooranna and Saggerson, 1976b). Also a lower concentration of adrenalin produced significant decreases in GPAT activity while only producing modest accumulation of unesterified FFA's (Sooranna and Saggerson, 1976a). The effect of adrenalin can be abolished by propanolol implying involvement of a β adrenergic receptor.

Nimmo and Houston (1978) have reported a 87% inhibition of GPAT activity by cAMP-dependent protein kinase when added to adipose tissue microsomes. GPAT activity could be reactivated to 80% of the previous activity by addition of alkaline phosphatase. No inhibition was recorded in the absence of ATP/$MgCl_2$ indicating inhibition by phosphorylation due to cAMP-dependent protein kinase. Unlike hormone sensitive lipase, GPAT activity could not be reactivated by addition of the multifunctional protein phosphatase III from rabbit muscle. Although GPAT can be reactivated by addition of alkaline phosphatase, no fraction of adipose tissue has been shown to be capable of reversing the reaction. These results serve to strengthen the analogies between TG synthesis and glycogen synthesis.

1.5.2 Monoacylglycerol-3-phosphate acyltransferase (MAGPAT)

This enzyme catalyses the second acylation step



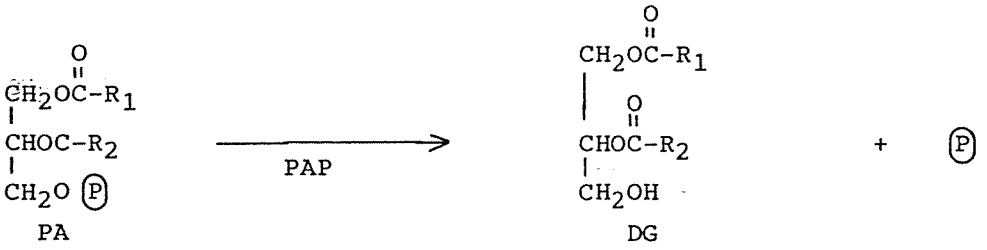
lysophosphatidic acid phosphatidic acid

An analysis of TG's of animal fats reveals that the positional distribution of FA's within the glyceride molecule is not random. There is a tendency for unsaturated FA's to occupy the sn-2 position and for saturated FA's to occupy either the sn-1 or the sn-3 position or both. The exception to this pattern is found in the pig where in all tissues, except the liver, 82% of the 16:0 is found in the sn-2 position (Mattson et al., 1964). The structure of the tissue TG's can be accounted for by the acyl donor specificity of the

microsomal acyltransferases. Although partially purified GPAT shows a preference for 16:0 compared to 18:1/18:2, MAGPAT shows no preference for unsaturated acyl-CoA donors. In conjunction the characteristic accumulation of short chain FA's (C -C) at the sn-3 position in ruminant milk TG's is most likely due to the ability of DGAT to preferentially use these short chain FA's (Marshall and Knudsen, 1977). Although acylation of G-3-P leads to PA as the major isolatable intermediate, MAGPAT is a distinct identity. All three acyltransferase activities have been partially separated from microsomes, solubilised with triton X-100, by sucrose density centrifugation (Hosaka et al., 1977).

1.5.3 Phosphatidic acid phosphatase (PAP)

Phosphatidic acid phosphatase (EC 3.1.3.4) catalyses the conversion of phosphatidic acid into diacylglycerol.

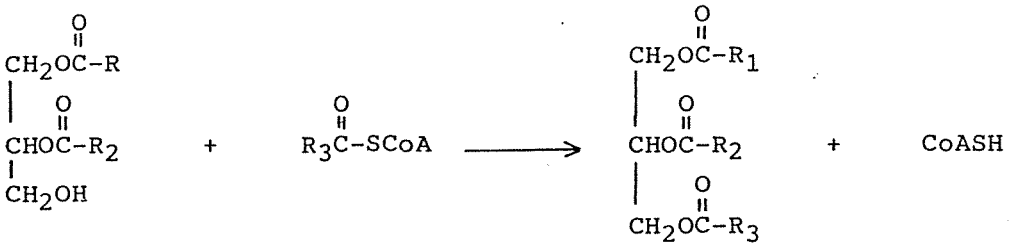


Studies of the incorporation of radioactive G-3-P into lipid show a rapid accumulation of radioactivity into PA with a much slower accumulation into neutral lipid (Fallon et al., 1975). As such PAP is considered to be the rate-limiting step in TG synthesis (Dodds et al., 1976a). Both Mg -dependent and Mg -independent forms of PAP have been shown to be present in the soluble and microsomal fractions of adipose tissue (Jamdar and Fallon, 1973). The specific activity of PAP is 20-fold higher in adipocytes compared to the liver and is the only activity of the TG synthetic pathway that does not increase with differentiation of 3T3-L1 preadipocytes

(Coleman et al., 1978). Adipocyte Mg - dependent PAP activity can be decreased by physiological concentrations of noradrenalin (Cheng and Saggerson, 1978a). This effect can be abolished by propanolol and insulin, with insulin resulting in an actual increase in PAP activity. Part of the inactivation of PAP activity by adrenalin could be due to an accumulation of FFA's, however, it cannot be considered secondary to the accumulation of FFA's (Sooranna and Saggerson, 1978c). It is therefore not known whether inhibition is due to some product of lipolysis or a more direct effect of the hormone, i.e., through a second messenger(s).

1.5.4 Diacylglycerol acyltransferase(DGAT)

The final enzyme in the TG biosynthetic pathway, DGAT (EC 2.3.1.20) catalyses the reaction



sn-1,2 diacylglycerol triacylglycerol

DGAT activity was first described from chicken adipose tissue (Weiss, et al. 1960). DGAT activity is present in a variety of tissues with greatest activity in adipose tissue and in liver (Table 1-1). The reaction catalysed by DGAT is the only reaction of the glycerolipid pathway that is exclusively concerned with TG synthesis. As the sn-1,2 DG substrate is also utilised in PL synthesis regulation of TG/PL synthesis at this branch point seems likely. Regulation of DGAT in adipose tissue seems likely since its uncontrolled action during lipolysis would form a futile cycle. It has been demonstrated by Brooks et al. (1982) that there is an increase in FA/TG cycling with the use of lipolytic hormones. DGAT activity has been documented from a

TABLE 1-1

Tissue survey of DGAT activity

Tissue	Specific activity
	nmoles min^{-1} mg $protein^{-1}$
Fat	51.3±20.1*
Liver	2.9
Brain	2.2
Lung	1.3
Intestine	1.2
Kidney	1.1
Heart	0.9
Skeletal muscle	0.4

* mean value ± S.D. of six independent microsomal preparations

Coleman and Bell (1976)

variety of tissues, namely rat liver (Hosaka, 1977; Haagsmann, et al 1982), rat adipocytes (Coleman and Bell, 1976), bovine mammary gland (Marshall and Knudsen, 1977) and pig perinephric adipose tissue (Stokes, et al. 1975). Characterisation of DGAT activity has always been impeded due to the difficulties in presenting DG substrates to the enzyme. Results from early methods have been proved unsatisfactory with the advent of an assay utilising ethanol as a dispersal medium (Coleman and Bell, 1976). The use of ethanol dispersal has revealed that detergents such as Tween 20 actually inhibit DGAT activity. There has been a 12-fold increase in DGAT activity in spinach leaves using a 0.02% solution of Zwittergent 2.08 rather than ethanol (Martin and Wilson, 1983). The use of ethanol-dispersed substrates has allowed the use of 100 to 1000-fold less protein than previous methods and results in a 100-fold increase in specific activity. This is essential as it has been shown that adipocytes contain little protein (Jarett, 1974). Adipose tissue DGAT has been characterised fully by Coleman and Bell (1976) in regard to substrate specificity. DGAT is highly specific for the sn-3 position as less than 5% of the activity present with sn 1,2 diolein as substrate was seen when using sn-1,3 diolein as the DG substrate. The dependence on the acyl-CoA substrates was also tested, with C_{10}-CoA giving the highest activity of all the acyl-CoA's tested.

1.6 Regulation of DGAT

Haagsman (1981a) has demonstrated that in hepatocytes exposed to glucagon there is a decrease in DGAT activity while cholinephosphotransferase activity is uneffected. While incubation of hepatocytes in the presence of FFA's leads to enhanced TG synthesis with

PL synthesis being less effected (Haagsman, 1981b). Hepatic DGAT activity can be reduced rapidly by incubation of microsomes in the presence of the 105,000 x g supernatant. Haagsman et al. (1982) concluded that this would tend to suggest that DGAT activity could be modulated in a reversible way, possibly by a phosphorylation-dephosphorylation mechanism similar to that proposed by Nimmo (1978) for the regulation of GPAT activity. This is supported by the fact that ATP cannot be replaced by methylene ATP, suggesting the cleavage of a phosphate group playing a role in the regulatory process. The use of fluoride ions in the homogenisation medium, which is a known inhibitor of phosphoprotein phosphatase, results in a lower DGAT activity. The activating factor has been shown to be a protein and the fact that it can be stored at $4^{o}C$ with no loss in activity eliminates soluble phosphatidic acid phosphatase which looses activity when stored at $4^{o}C$ (Haagsman, et al. 1982). This process of reversible activation/inactivation would therefore allow for rapid interconversion of DGAT between metabolically active and inactive forms. Apart from this reported control of DGAT, the enzyme is also subject to control by a number of metabolic effectors with levels of acyl-CoA substrates being inhibitory above 40 μM (Coleman and Bell, 1976). Conversion of DG to TG has been reported to be activated 15-fold by Z protein which constitutes 2% of liver proteins and is known to have different affinities for the various acyl-CoAs and is thought to actively promote the activity of DGAT.

1.7 Systems for the study of TG biosynthesis in adipose tissue

Many approaches have been taken in the course of the study of the TG biosynthetic pathway. These include use of whole adipose tissue (incubation of the fat pad), whole adipose tissue homogenates, isolated

adipocytes, subcellular fractionation (microsomal vesicles) and use of the fat cell model (3T3-L1 preadipocyte cell line). Tissue homogenates have probably been the most commonly used source of enzymes in the investigation of TG synthesis. This cell-free system, which contains the whole complement of synthetic enzymes, has been very important in determining the steps involved. Information about the possible control of the pathway and characterisation of cofactor requirements has been gained through use of this system (Christie et al., 1976). Isolated adipocytes make use of the isolated functional adipose tissue unit and allows the role of transport processes to be studied, especially the effects of hormones which normally interact at the plasma membrane. Incubation of adipocytes with hormones followed by homogenisation and study of the individual enzyme activities has lead to the elucidation of the regulatory steps of the synthetic pathway (Sooranna and Saggerson, 1976a,b; 1978a,b,c). Subcellular fractionation (Jarett, 1974) involves the isolation of the microsomal fraction which contains the complement of enzymes associated with the endoplasmic reticulum (Ballas and Bell, 1976). Higher specific activities of the enzymes are achieved, but there are difficulties in presenting the substrates to the enzyme system. The 3T3-L1 subline of the original Swiss 3T3-M fibroblasts has the capacity to differentiate into adipocyte colonies (Green and Kehinde, 1974). During the differentiation of 3T3-L1 fibroblasts to adipocytes there is a simultaneous and coordinated increase in the activity of the four microsomal enzymes of TG synthesis; namely acyl-CoA synthetase, GPAT, MAGPAT and DGAT. The specific activities of these enzymes were 30-100-fold greater compared to undifferentiated 3T3-L1 or 3T3-C2 control cells (Coleman et al. 1978). The levels of PAP activity best correlates the TG content of the cell while DGAT

activity is a sensitive indicator of 3T3-L1 differentiation into adipocytes (Grimaldi et al., 1978). The rise in enzyme activities during differentiation can be prevented by cycloheximide indicating induction due to increased enzyme synthesis.

The 3T3-L1 fat cell model can also be used to follow the development of hormone receptors and hormone responsiveness in adipocytes (Rosen et al., 1978). Whichever system is used to study TG systhesis a number of difficulties exist, these include; the tight association of enzymes with the endoplasmic reticulum resulting in difficulties in solubilisation, difficulty in fractionation of solubilized enzymes, the dependence of partially purified enzymes on PL's for activity and the delivery of amphipathic and hydrophobic substrates to membrane bound and solubilised enzyme preparations. Therefore our understanding of the regulatory process of the glycerolipid pathway has been limited due to the lack of homogenous enzymes, specific enzyme inhibitors and mutations containing defective enzymes.

1.8 The effect of adipocyte size

It is a well established fact that the size (surface area) of fat cells from man and animals is an important determinant for metabolic rates (Jamdar and Osborne, 1981). Those functions of adipose tissue which are subject to change with adipocyte cell size include lipolysis, lipoprotein lipase activity, glucose incorporation into lipid and esterification of exogenous FFA's into lipid. Glycerolipid formation is most active in rat adipose tissue taken from donors 60 days old where the mean adipocyte cell size is 66 μM, compared to 74 μM in animals 130 days old (Jamdar and Osborne, 1981). The accompanying increase in the amount of adipose tissue with age is known to be due to both hypertrophy

and hyperplasia of the adipocyte (Hirsh and Han, 1969). In age-dependent studies glycerolipid formation was shown to be low in 15 day-old animals with a 6-7-fold increase at 30 days with maximal activity at 60 days. The decline with further age has been linked with age, as adipocytes taken from animals 28 days old were twice as active in glyceride formation as adipocytes of the same size taken from 100 day-old animals (Jamdar and Osborne, 1981). It has been intimated that both PAP and DGAT activities may be higher in younger rats (30 days) compared to older rats (120 days old). Changes in enzyme activity with age could be due to variations in the PL content of the microsomal membranes; several of the TG synthetic enzymes require PL's for their activity (Coleman and Bell, 1980). In conjunction Jamdar et al., (1981) has reported an increase in GPAT activity with age (15 to 60 days) and also a 3.5-fold increase in DGAT activity, and concludes that there is an acceleration of the entire pathway with age. Also of importance is the anatomical location of the adipocytes, with subcutaneous adipocytes being significantly smaller than perirenal and epididymal adipocytes.

1.9 The effects of hormones on triacylglycerol synthesis

When assessing the effects of hormones it is difficult to decide whether the changes are due to alterations in intracellular enzyme activities or are they secondary to changes in precursor concentrations in the adipocyte. The hormonal control of TG synthesis has been summarised by Nimmo (1980) while the effects of hormones on the enzymes of lipid metabolism have been summarised by Saggerson et al. (1979).

1.9.1 The effects of insulin

The effects of insulin appear to be initiated by the interaction of the hormone with its receptor on the target cell plasma membrane (Butcher, et al 1973). The binding of insulin to its receptor correlates with the initiation of many of the short term effects of insulin including stimulation of glucose transport, stimulation of glucose metabolism, lipogenesis and inhibition of lipolysis. Phosphorylation of proteins has been shown to be an important regulatory mechanism in metabolic pathways (Greengard, 1978). Treatment of adipocytes with insulin has been shown to alter the incorporation of ^{32}P, phosphate into several phosphoproteins (Benjamin and Clayton, 1978) (Walaas, et al. 1981). Control of membrane protein phosphorylation may be of importance in the initial steps of insulin action. Hormone control of the phosphorylation of serine residues could alter specific properties of the proteins controlling the entry of solute molecules into the channel and hence could alter the membranes transport capacity.

In isolated adipocytes insulin treatment results in an increase in TG synthesis (Sooranna and saggerson, 1975). This is probably due to an increase in glucose transport into the adipocyte and thus increasing the amount of triose phosphate available for esterification. Coupled to this is the strong effect of insulin on lipoprotein lipase which increases the supply of exogenous precursors to fat cell. Insulin also effects a 2-fold increase in acyl-CoA synthetase activity increasing the supply of acyl CoA thioesters (Jason et al., 1976).

Insulin has been shown to stimulate fatty acid synthesis at low concentrations of fatty acids, probably through activation of acetyl-CoA carboxylase and pyruvate dehydrogenase (Sooranna and Saggerson, 1975). Insulin inhibits lipolysis in adipocytes at physiological concentrations

(1 nM). The mechanism by which insulin achieves this may be due to a lowering of cAMP levels, but the decrease in lipolysis is not comparable to the increase in cAMP levels (Kono, 1973). When the levels of cAMP have been elevated by noradrenalin the relative effect of insulin to lower the nucleotide level is too small to explain the antilipolytic effect of the hormone. Treatment of rat adipocytes with insulin results in a small but significant activation of GPAT activity (Sooranna and Saggerson, 1976a). Insulin alone has no effect on PAP activity, it does however reverse the inactivation of PAP by noradrenalin (Cheng and Saggerson, 1978b). A similar effect is seen in DGAT activity where insulin alone has no effect but it can oppose the effects of catecholamines (Sooranna and Saggerson, 1978c).

1.9.2 The effects of adrenalin

In adipose tissue, adrenalin has a lipolytic effect with an increase in both the release of FFA's into the blood and the rate of their re-esterification back into TG. The majority of the effects of adrenalin can be blocked by propanolol indicating action via a β-adrenergic receptor. Adrenalin has been shown to cause; a 30-40% decrease in acyl-CoA synthetase activity (Sooranna and Saggerson, 1978), a dose-dependent decrease in GPAT activity (up to 50%) (Sooranna and Saggerson, 1978a) a decrease in the diolein dependent incorporation of 16:0 CoA into TG (Sooranna and Saggerson, 1978e). The net result of adrenalin action therefore is a decrease in the activity of the key regulatory sites of TG synthesis resulting in decreased TG synthesis. These effects have been observed in tissue that has been exposed briefly to adrenalin, freeze-stopped and then a tissue extract prepared for the enzyme assay. This method gives insight into the location of the individual steps that are altered by adrenalin, but does not reveal anything about the mechanisms involved.

1.9.3 The effects of noradenalin

Much less is known about the action of noradrenalin on adipose tissue compared to the actions of insulin and adrenalin. Incubation of adipocytes with noradrenalin causes a rapid inactivation of PAP activity. This inactivation is confined to the Mg^{++}-dependent activity which is mainly in the soluble fraction (Cheng and Saggerson, 1978a). An important governing factor appears to be the ratio of catecholamines to insulin.

1.9.4 The effects of glucagon

The concentration of glucagon, a lipolytic hormone, increases with fasting and corresponds to a decrease in FA synthesis and an increase in FA oxidation. Inhibition of glycerolipid formation by glucagon is probably best explained by an inhibition of glycolysis, this limits the supply of triose phosphate precursors. In liver glucagon has been shown to enhance the utilisation of DG's for PL synthesis, this may be important in maintaining the rate of PL synthesis at the expense of TG formation (Geelen et al., 1978a).

1.10 Effect of diet on triacylglycerol synthesis

It is expected that the rate of TG synthesis will be high in both the liver and adipose tissue of animals that consume large amounts of fat and carbohydrate. Conversely during starvation TG is broken down in adipose tissue and the FA's transported to the liver and the other organs for metabolism. It is therefore reasonable to assume that these dietary changes will be reflected by changes in the activities of the TG synthetic enzymes. The effects of these dietary modifications on the hepatic activity of the enzymes of TG synthesis have been summarised (Brindley, 1978).

1.10.1 The effect of starvation

During periods of starvation there is a decrease in the amount of adipose tissue and a reduction in the rate of TG formation by adipose tissue homogenates (Angel and Roncari, 1967). This reduced ability to synthesise TG should therefore be reflected in the reduced activity of one or more of the synthetic enzymes. A 20% reduction in GPAT activity in adipocyte homogenates has been recorded during a 48hr fast (Sooranna and Saggerson, 1979), as well as a 64% reduction in cytosolic Mg^{++}-dependent PAP activity during a 72hr fast (Moller et al., 1977). In conflict with these results Lawson et al., 1981) has reported no significant changes in GPAT, Mg^{++}- dependent PAP or DGAT activities over a 48hr fast. They did however note a 25% decrease in palmitoyl-CoA synthetase activity.

The products of esterification of G-3-P by adipocyte homogenates from animals subjected to starvation are altered in comparison to fed controls. The esterification of FA's into PA is increased with an accompanying decrease in the formation of neutral lipid, which is consistent with a decrease in PAP activity (Jamdar and Osborne, 1982). In adipose tissue from normally-fed animals the rate of oxidation of FA's is low compared to the rate of estification. During starvation there is a decrease in the ability to restrain the oxidation of FA's, both endogenous and exogenous (Harper and Saggerson, 1976). Refeeding rapidly restores lipid synthesis to the normal fed levels and frequently produces an overshoot (Etherton and Allen, 1980). The age of the adipose tissue also appears to influence the effect of starvation, with a 2-fold decrease in GPAT activity recorded in young rats (42-60 days) following a 72hr fast but no decrease recorded in older rats (120 days) even though they were fasted for 96hr. A 2-fold reduction in PAP

activity was seen regardless of tissue age (Jamdar and Osborne, 1982). Also in starvation there is a marked decrease in lipoprotein lipase activity and also a significant decrease in the supply of acyl-CoA thioesters available for esterification (Lawson et al., 1981).

1.10.2 The effect of a high lipid diet

Increasing the amount of lipid in the diet, either by the addition of corn-oil (polyunsaturated plant oil) or tallow (a more saturated animal fat), has the effect of increasing plasma levels of glucose, cholesterol and glycerol. Accompanying insulin levels are above normal, indicating perhaps a diminished sensitivity to insulin. The lipolytic effects of glucagon, adrenalin and noradrenalin are also reduced in fat-fed animals, the diminished response due to the reduced activation of the adenylate cyclase (Gorman et al., 1973). The binding of adrenalin to the receptors is not effected therefore the effect must be at a point between the receptor and the activation of adenylate cyclase. There is also a diminished response to insulin in animals on a high-fat diet. This mechanism of insulin insensitivity could be related to the effect of diet on the cell membrane (Gorman et al., 1973) and could explain the decrease in lipoprotein lipase activity in animals fed a high-fat diet (Lawson et al., 1981). These diminished cellular responses may be due to an increase in the size of the fat-cell (Gorman et al., 1972).

1.10.3 The effect of a high carbohydrate diet

Diets rich in carbohydrate increase the levels of plasma TG and can lead to triglyceridaemia (Smith et al., 1974). The greatest change in activity of the enzymes associated with TG synthesis is observed in lipoprotein lipase. A high carbohydrate diet leads to increased GPAT

activity and twice the molar flux through PAP compared to high lipid diets, this is compatible to the increased activities of PAP and DGAT (Dodds et al., 1976b). This is in agreement with a glucose/fructose diet increasing PAP/DGAT activity in the liver (Fallon et al., 1975). Generally a high carbohydrate diet leads to enhanced storage of the excess caloric material as TG.

1.10.4 The effect of a high-protein diet

The effect of a high protein diet on adipose tissue metabolism is largely unknown although its effects on hepatic glyceride formation have been documented (Holub, 1975). In the liver results indicate that the level of dietary protein can regulate the rate of lipid synthesis from G-3-P (Holub, 1975).