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A STUDY OF THE REGULATION OF  
HEPATIC MICROSOMAL GLYCEROL PHOSPHATE ACYLTRANSFERASE (GPAT)

A thesis presented in partial fulfilment of the  
requirements for the degree of Doctor of Philosophy  
in Biochemistry at  
MASSEY UNIVERSITY

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1986

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ABSTRACT

Experiments described in this thesis were conducted to examine the possibility that hepatic microsomal GPAT activity in rats is regulated by insulin.

Hepatic microsomal fractions were prepared by a procedure based on published methods and it was established by assay of cytochrome oxidase, monoamine oxidase and NADPH cytochrome C reductase that there was less than 11% mitochondrial impurity. A butanol extraction of GPAT assays was adopted to separate the butanol-soluble [ $^{14}\text{C}$ ]-lipid products from the unreacted aqueous-soluble [ $^{14}\text{C}$ ]-glycerol 3-phosphate substrate. Methods were developed to simplify the determination of [ $^{14}\text{C}$ ]-radioactivity. The kinetics of the response of the assay system to changes in the concentrations of glycerol 3-phosphate and palmitoyl-CoA were similar to those published for the microsomal GPAT. The products of the assay were identified as phosphatidic acid and lysophosphatidic acid by their chromatographic properties before and after hydrolysis with chicken liver phosphatidate phosphohydrolase. These products are consistent with literature reports.

Male Sprague-Dawley rats were treated with insulin (4 i.u./kg body weight) or saline, and extracts of hind-limb muscle were prepared and fractionated on Sephadex G-25 in 50 mM formic acid. Fractions which eluted subsequent to the void volume were assayed with hepatic microsomal GPAT and the effect of insulin-treatment fractions were compared with the effect of saline-treatment fractions.

Fractions containing material of approximately 3000 and 1000 daltons molecular weight enhanced GPAT activity in an insulin-dependent manner by 0.46 and 0.64 nmol/min/mg of microsomal protein, respectively (both  $P < 0.01$ ), compared to the effect of the saline controls. Control rates were approximately 3.5 nmol/min/mg of microsomal protein. It was calculated that these insulin-dependent increases in hepatic microsomal GPAT activity would be sufficient to account for the difference between the estimated hepatic triacylglycerol production of fed and fasted rats. Furthermore, published studies suggest that insulin-dependent changes in activities of enzymes, demonstrated with in vitro systems utilising low molecular weight fractions from rat muscle, may parallel sensitivity of the same enzymes to insulin in vivo. The low molecular weight stimulator or stimulators of hepatic microsomal GPAT have an apparent molecular weight within the range 1000-3000 daltons, appear to be heat and acid stable, are soluble in aqueous solution, have very low absorbance at 220nm (or a very high specific activity) and may be sensitive to oxygen. These properties suggest that the low molecular weight stimulator or stimulators of hepatic microsomal GPAT activity may be related to the putative insulin mediator substance (IMS).

In initial experiments, where rats were heparinised prior to treatment with insulin or saline, it was observed that some fractions were able to stimulate hepatic microsomal GPAT activity in an insulin-independent manner. Experiments to resolve this suggested that the treatment of rats with heparin alone led to the presence of low molecular weight material, in the fractions of muscle extracts, with the potential to enhance GPAT activity. It was found that low

molecular weight fractions of the saline treatment muscle extracts did not enhance GPAT activity. This supported the suggestion that heparin was responsible for the ability of low molecular weight fractions of muscle extracts to stimulate GPAT activity in an insulin-independent manner.

Experiments were also conducted in which impure hepatic plasma membranes were treated with insulin (20-1000  $\mu$ units/ml). However, when hepatic microsomal GPAT was assayed with material from these incubations a stimulator of GPAT was not detected.

The results of experiments presented in this thesis provide further evidence in favour of the hypothesis that hepatic microsomal GPAT activity can be modified by insulin and may contribute to the overall regulation of glycerolipid biosynthesis in liver.

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TABLE OF CONTENTS

	<u>Page</u>
ABSTRACT.....	i
ACKNOWLEDGEMENTS.....	iv
TABLE OF CONTENTS.....	v
LIST OF FIGURES.....	xi
LIST OF TABLES.....	xv
LIST OF ABBREVIATIONS.....	xix
LIST OF APPENDICES.....	xxi
<u>Chapter 1.</u> <u>INTRODUCTION</u> .....	1
1.1 THE CENTRAL ROLE OF GLYCEROLIPIDS IN METABOLISM.....	2
1.2 PATHWAYS FOR THE SYNTHESIS OF GLYCEROLIPIDS.....	7
1.3 THE CONTROL OF GLYCEROLIPID BIOSYNTHESIS.....	15
1.3.1 REGULATION BY SUBSTRATE AVAILABILITY.....	15
1.3.2 REGULATION BY CONTROL OF PHOSPHATIDATE PHOSPHOHYDROLASE ACTIVITY.....	23
1.3.3 REGULATION BY CONTROL OF DIACYLGLYCEROL ACYLTRANSFERASE ACTIVITY.....	28
1.3.4 REGULATION BY CONTROL OF GLYCEROL PHOSPHATE ACYLTRANSFERASE ACTIVITY.....	31
1.3.4.1 Physiological effectors of GPAT activity.....	31
1.3.4.2 Dietary effectors of GPAT activity.....	34
1.3.4.2.1 Fasting.....	35
1.3.4.2.2 High fat, high carbohydrate and ethanol diets.....	37



TABLE OF CONTENTS (continued)

	<u>Page</u>
1.3.4.3 Pharmacological effectors of GPAT activity.....	38
1.3.4.4 Control by glucagon, catecholamines and insulin.....	39
1.3.4.5 Control by other hormones.....	43
1.4 GLYCEROLPHOSPHATE ACYLTRANSFERASE - CHARACTERISTICS AND PROPERTIES.....	45
1.4.1 Distribution of GPAT between mitochondrial and microsomal fractions.....	45
1.4.2 Location of GPAT within mitochondrial and microsomal fractions.....	47
1.4.3 Properties of GPAT.....	48
1.4.4 GPAT purification.....	59
1.5 PURPOSE AND SCOPE OF THE INVESTIGATION.....	62
<u>Chapter 2.</u> <u>MATERIALS</u> .....	63
2.1 Reagents.....	64
2.2 Instruments & Equipment.....	64
2.3 Statistical package.....	64
2.4 Animals.....	64
2.4.1 Rats.....	64
2.4.2 Chickens.....	65

TABLE OF CONTENTS (continued)

	<u>Page</u>
<u>Chapter 3.    METHOD DEVELOPMENT AND VALIDATION OF GPAT</u>	
<u>ASSAY PROCEDURE</u> .....	68
3.1 Introduction.....	69
3.2 Procedure for subcellular fractionation.....	70
3.3 Validation of subcellular fractionation procedure.....	73
3.4 Method for protein determination.....	82
3.5 Determination of radioactivity.....	83
3.6 Extraction of radioactive glycerol 3-phosphate from aqueous butanol.....	85
3.7 Examination of microsomal esterification assay system.....	87
3.7.1 Preparation of glycerol 3-phosphate and palmitoyl-CoA.....	87
3.7.2 Initial selection of conditions for GPAT assay.....	87
3.7.3 Response to [palmitoyl-CoA].....	89
3.7.4 Response to [glycerol 3-phosphate].....	90
3.7.5 Response to amount of microsomal protein.....	99
3.7.6 Dependence on time.....	99
3.7.7 Response to pH.....	99
3.7.8 Identification of products of the GPAT assay.....	105
3.7.8.1 Thin layer chromatography.....	105
3.7.8.2 Protocol for identification radioactive products of the microsomal GPAT reaction.....	106
3.8 Summary of methodology adopted for the assay of GPAT.....	115

TABLE OF CONTENTS (continued)

	<u>Page</u>
<u>Chapter 4. EXPERIMENTS TO EXAMINE THE EFFECT OF INSULIN</u>	
<u>ON GPAT ACTIVITY</u> .....	116
4.1 Introduction.....	117
4.2 Methods.....	119
4.2.1 Effect of insulin on blood glucose.....	119
4.2.2 General method for preparation of rat muscle extracts.....	119
4.2.3 Chromatography of extracts.....	121
4.2.4 Assay of Sephadex G-25 fractions with liver microsomal GPAT.....	123
4.3 Results and Discussion.....	128
4.3.1 Section 1 - Preliminary experiments with extracts from heparinised rats treated with insulin (Extract A) or saline (Extract B), respectively: the effect of low molecular weight fractions of these extracts on GPAT activity.....	128
4.3.2 Section 2 - Experiments with further extracts from heparinised rats treated with insulin (Extracts C, D, E, & G) or saline (Extracts F & H): reconsideration of the role of liquid air versus liquid nitrogen in the method for preparing extracts...	145

TABLE OF CONTENTS (continued)

	<u>Page</u>
4.3.3 Section 3 - Experiments with extracts from 48 h-fasted heparinised rats treated with insulin (Extract K) or saline (Extracts I & J), and with Extracts L and M from fed or 48 h-fasted non-cannulated rats, respectively: consideration of the possible influence of cannulation and heparin.....	163
4.3.4 Section 4 - Experiments with extracts from non heparinised rats treated with insulin (Extracts N, P, & R) or saline (O, Q & S): insulin-dependent stimulation of hepatic microsomal GPAT activity.....	183
 <u>Chapter 5. THE EFFECT OF SUPERNATANTS FROM LIVER</u>	
<u>PARTICULATE FRACTIONS ON GPAT.....</u>	189
 5.1 Introduction.....	190
5.2 Methods.....	193
5.2.1 Selection of method for preparation of liver particulate fraction enriched in plasma membranes.....	193
5.2.2 Incubation of particulate fraction with insulin or buffer.....	197
5.2.3 Assay to examine the effect of particulate fraction supernatant on GPAT activity.....	197
5.3 Results and Discussion - The effect of supernatants from liver particulate fractions treated with insulin.....	199

TABLE OF CONTENTS (continued)

	<u>Page</u>
<u>Chapter 6. DISCUSSION AND CONCLUSIONS</u> .....	205
APPENDICES.....	228
BIBLIOGRAPHY.....	248

LIST OF FIGURES

<u>Figure</u>	<u>Page</u>
1.1: Enzymes and pathways of glycerolipid biosynthesis.....	5
3.1: Scheme for the subcellular fractionation of rat liver.....	71
3.2: The specific activity and total activity of cytochrome oxidase in various subcellular fractions.....	75
3.3: The specific activity and total activity of monoamine oxidase in various subcellular fractions.....	77
3.4: The specific activity and total activity of NADPH cytochrome C reductase in various subcellular fractions.....	79
3.5: Effect of [palmitoyl-CoA] on esterification of glycerol 3-phosphate with palmitoyl-CoA by a hepatic microsomal fraction.....	91
3.6: Hill plot transformation of the data for the response of esterification of glycerol 3-phosphate with palmitoyl-CoA by a hepatic microsomal fraction to varying [palmitoyl-CoA].....	93
3.7: Effect of [glycerol 3-phosphate] on esterification of glycerol 3-phosphate with palmitoyl-CoA by a hepatic microsomal fraction.....	95
3.8: Eadie-Hofstee transformation of data for the effect of [glycerol 3-phosphate] on esterification of glycerol 3-phosphate with palmitoyl-CoA by a hepatic microsomal fraction.....	97

LIST OF FIGURES (continued)

<u>Figure</u>	<u>Page</u>
3.9: Effect of amount of microsomal protein on esterification of glycerol 3-phosphate with palmitoyl-CoA by a hepatic microsomal fraction.....	100
3.10: Effect of time on esterification of glycerol 3-phosphate with palmitoyl-CoA by a hepatic microsomal fraction.....	102
3.11: Identification of microsomal GPAT assay products - Part 1.....	109
3.12: Identification of microsomal GPAT assay products - Part 2.....	113
4.1: The effect of insulin on blood glucose concentration.....	124
4.2: Titration curve of rat muscle solution acidified with acetic acid to pH 3.8.....	126
4.3: Sephadex G-25 fractionation of muscle extract from insulin-treated, heparinised rat and absolute effect of fractions on microsomal GPAT from livers of fed or fasted rats.....	136
4.4: Sephadex G-25 fractionation of muscle extract from heparinised rats treated with insulin and saline and absolute effect of fractions on microsomal GPAT from livers of fed rats.....	139

LIST OF FIGURES (continued)

<u>Figure</u>	<u>Page</u>
4.5: Sephadex G-25 fractionation of muscle extract (twice usual loading on column) from heparinised rat treated with insulin and absolute effect of fractions on microsomal GPAT from livers of fed rats.....	142
4.6: Sephadex G-25 fractionation of muscle extract from a heparinised rat treated with insulin and absolute effect on GPAT activity: inhibition of GPAT activity relative to the formic acid control GPAT activity.....	148
4.7: Sephadex G-25 fractionation of muscle extract from heparinised rats treated with insulin or saline and absolute effect on GPAT: inhibition of GPAT activity relative to formic acid controls.....	151
4.8: Sephadex G-25 fractionation of muscle extract from heparinised rats treated with insulin or saline and absolute effect on GPAT: inhibition of GPAT activity relative to formic acid controls.....	153
4.9: The absolute effect of Sephadex G-25 fractions of muscle extracts from heparinised rats treated with insulin or saline, prepared using liquid nitrogen, on microsomal GPAT from livers of fed rats.....	159
4.10: The absolute effect of Sephadex G-25 fractions of muscle extract from 48 h-fasted heparinised rat treated with saline on microsomal GPAT from fed rat.....	168



LIST OF FIGURES (continued)

<u>Figure</u>	<u>Page</u>
4.11: The absolute effect of Sephadex G-25 fractions of muscle extract from 48 h-fasted heparinised rats treated with saline or insulin on microsomal GPAT from fed rats.....	170
4.12: The absolute effect of Sephadex G-25 fractions of muscle extract from fed, non-cannulated rat, on microsomal GPAT from fed rat.....	178
4.13: The absolute effect of Sephadex G-25 fractions of muscle extract from 48 h-fasted, non-cannulated rat on microsomal GPAT from fed rat.....	180
4.14: The absolute effect of Sephadex G-25 fractions of muscle extract from fed non heparinised rats treated with either insulin or saline, on microsomal GPAT from livers of fed rats.....	186
Appendix I	
I.1: Elution of carbohydrate, as shown by Phenol-sulphuric reactivity, in Sephadex G-25 fractions of muscle extracts from heparinised rats treated with either insulin or saline.....	233

LIST OF TABLES

<u>Table</u>	<u>Page</u>
1.1: Treatments reported to affect glycerol 3-phosphate concentrations in mammals.....	16
1.2: Concentrations of glycerol 3-phosphate in tissues of fed mammals.....	18
1.3: Concentrations of glycerol 3-phosphate in tissues of fasted rats.....	20
1.4: Hepatic mitochondrial GPAT - $K_m$ values for glycerol 3-phosphate.....	54
1.5: Microsomal GPAT - $K_m$ values for glycerol 3-phosphate.....	55
2.1: Reagents used and source of supply.....	66
2.2: Reagents used and source of supply. ....	67
3.1: Protein content and marker enzyme activities in subcellular fractions of rat liver.....	81
3.2: The effect of using the Automatic Quench Compensation facility of the Beckman LS 8000 scintillation counter on the efficiency of counting of [ $^{14}\text{C}$ ]-hexadecane in the presence of varying amounts of water-saturated butanol.....	84
3.3: The effect of the volume of the aqueous phase and number of washings on the removal of [ $^{14}\text{C}$ ]glycerol 3-phosphate from aqueous butanol.....	86
3.4: The effect of pH of the reaction on esterification of glycerol 3-phosphate with palmitoyl-CoA by a hepatic microsomal fraction.....	104

LIST OF TABLES (continued)

<u>Table</u>	<u>Page</u>
4.1: The effect of Sephadex G-25 fractions of muscle extract (A) from heparinised rat treated with insulin on liver microsomal GPAT from fed rats or 72 h-fasted rats.....	138
4.2: The effect of Sephadex G-25 fractions of muscle extract from heparinised rats treated with insulin (Extract A) or saline (Extract B) on liver microsomal GPAT from fed rats.....	141
4.3: The effect of Sephadex G-25 fractions of muscle extract (A) from a heparinised rat treated with insulin on liver microsomal GPAT from fed rats.....	144
4.4: The effect of Sephadex G-25 fractions of muscle extract (C) from a heparinised rat treated with insulin, prepared using liquid air, on liver microsomal GPAT from fed rat (Experiment 6).....	150
4.5: The effect of Sephadex G-25 fractions of muscle extracts from heparinised rats treated with insulin (Extracts D & E) or saline (Extract F), prepared using liquid air, on liver microsomal GPAT from fed rats.....	155
4.6: The effect of Sephadex G-25 fractions of muscle extracts from heparinised rats treated with insulin <sup>d</sup> (means of values in Fig 4.5) or saline <sup>e</sup> , prepared using liquid air, on microsomal GPAT from fed rats.....	156

LIST OF TABLES (continued)

<u>Table</u>	<u>Page</u>
4.7: The effect of Sephadex G-25 fractions of muscle extracts, from heparinised rats treated with insulin (Extract G) or saline (Extract H), using liquid nitrogen, on liver microsomal GPAT from fed rats.....	161
4.8: The absolute and percentage effect of Sephadex G-25 fractions of muscle extracts from heparinised rats treated with insulin (Extract G) or saline (Extract H), prepared using liquid nitrogen, on liver microsomal GPAT from fed rats.....	162
4.9: The effect of Sephadex G-25 fractions of muscle extracts from 48 h-fasted heparinised rats treated with saline (Extracts I & J) on liver microsomal GPAT from fed rats.....	172
4.10: The effect of Sephadex G-25 fractions of muscle extracts from 48 h-fasted heparinised rats treated with saline (Extracts I & J) or insulin (Extract K) on liver microsomal GPAT from fed rats.....	173
4.11: The effect of Sephadex G-25 fractions of muscle extracts from fed or 48 h-fasted, non-cannulated rat on liver microsomal GPAT from fed rats.....	182
4.12: The absolute and percentage effects of Sephadex G-25 fractions of muscle extracts from fed rats treated with either insulin or saline, on microsomal GPAT from livers of fed rats.....	188
5.1: Results for 5'-nucleotidase marker study using Method 3 for preparation of liver particulate fraction.....	195

LIST OF TABLES (continued)

<u>Table</u>	<u>Page</u>
5.2: The effect of supernatant from liver particulate fraction treated with 20, 100 or 500 $\mu$ units of insulin/ml on GPAT.....	203
5.3: The effect of supernatant from liver particulate fraction treated with 500 or 1000 $\mu$ units insulin per ml on liver microsomal GPAT.....	204
<u>Appendices</u>	
I.1: The effect of fructose 2,6-bisphosphate on microsomal GPAT.....	236
II.1: The effect of ATP, ADP, and AMP on hepatic microsomal GPAT from fed rats.....	242
III.1: The effect of oestradiol 17 $\beta$ -dipropionate on chicken liver microsomal GPAT activity.....	247

LIST OF ABBREVIATIONS

ADP	adenosine diphosphate
AMP	adenosine monophosphate
cAMP	adenosine 3',5'-cyclic monophosphate
AQC	Automatic Quench Compensation
ATP	adenosine triphosphate
BSA	bovine serum albumin
CoA	coenzyme A
cpm	counts per minute
dpm	disintegrations per minute
DG	diacylglycerol
DHAP	dihydroxyacetone phosphate
DHAPAT	dihydroxyacetone phosphate acyltransferase
DTNB	dithiobis nitrobenzoic acid
EDTA	ethylene diamine tetraacetic acid
$\alpha$ -GP or GP	<u>sn</u> -glycerol 3-phosphate
GPAT	acyl-CoA: <u>sn</u> -glycerol 3-phosphate acyltransferase
h	hour(s)
IMS	insulin mediator substance
i.u.	international units
LPA	lysophosphatidic acid
min	minute(s)
MG	monoacylglycerol
NEM	N-ethylmaleimide
NADH	$\beta$ -nicotinamide adenine dinucleotide, reduced

LIST OF ABBREVIATIONS (continued)

NADPH	nicotinamide adenine dinucleotide phosphate, reduced
PA	phosphatidic acid
PC	phosphatidylcholine
PL	phospholipid
POPOP	1,4-bis[2-(5-phenyloxazolyl)]benzene
PPO	2,5-diphenyloxazole
sec	seconds
SD	standard deviation
SEM	standard error of the mean
TLC	thin-layer chromatography
TMPD	tetra methyl phenylene diamine
Tris	tris-(hydroxymethyl)-aminomethane
VLDL	very low density lipoprotein
v/v	volume/volume
v/w	volume/weight
vol	volume(s)

NOTE

In this thesis unless stated otherwise, < 100% = less than control enzyme activity; 100% = control enzyme activity; > 100% = stimulation of enzyme activity relative to the appropriate control.

LIST OF APPENDICIES

	<u>Page</u>
Appendix I. THE EFFECT OF FRUCTOSE 2,6-BISPHOSPHATE ON GPAT.....	228
Appendix II. THE EFFECT OF ADENINE NUCLEOTIDES ON GPAT.....	239
Appendix III. THE EFFECT OF OESTROGEN ON CHICKEN LIVER MICROSOMAL GPAT.....	243