# 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

EARL VICTOR JOHN STEVENS

1986

MASSEY UNIVERSITY

1. (a) I give permission for my thesis, entitled

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

to be made available to readers in the Library under the conditions determined by the Librarian.

(b) I agree to my thesis, if asked for by another institution, being sent away on temporary loan under conditions deterined by the Librarian.

(c) I also agree that my thesis may be copied for Library use.

~~2. I do not wish my thesis, entitled~~

~~to be made available to readers or to be sent to other institutions without my written consent within two years.~~

Signed E.V.J. Stevens

Earl Victor John STEVENS

Date 24/1/86

Strike out the sentence or phrase which does not apply.

The Library,
Massey University,
Palmerston North, N.Z.

The copyright of this thesis belongs to the author. Readers must sign their name in the space below to show that they recognise this. They are asked to add their permanent address.

## 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 [$^{14}$C]-lipid products from the unreacted aqueous-soluble [$^{14}$C]-glycerol 3-phosphate substrate. Methods were developed to simplify the determination of [$^{14}$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 μunits/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.

## ACKNOWLEDGEMENTS

I wish to thank my supervisor, Dr D. R. Husbands, for his advice during the course of this study and also Dr J. C. Hawke for his helpful comments on aspects of the work. The help and encouragement received from friends and colleagues was appreciated. I would also like to acknowledge the encouragement of my wife Debbie and the support of my parents, Vic & Betty Stevens.

# TABLE OF CONTENTS

TABLE OF CONTENTS (continued)

TABLE OF CONTENTS (continued)

TABLE OF CONTENTS (continued)

TABLE OF CONTENTS (continued)

TABLE OF CONTENTS (continued)

# LIST OF FIGURES

LIST OF FIGURES (continued)

LIST OF FIGURES (continued)

LIST OF FIGURES (continued)

# LIST OF TABLES

LIST OF TABLES (continued)

LIST OF TABLES (continued)

LIST OF TABLES (continued)

# 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 |
| α-GP or GP | sn-glycerol 3-phosphate |
| GPAT | acyl-CoA: sn-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 | β-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