

科技资料

# **Diabetes, Obesity and Hyperlipidemias-IV**

# DIABETES, OBESITY AND HYPERLIPIDEMIAS - IV

Proceedings of the 5th European Symposium  
on Metabolism, Padova, 15-17 May 1989

*Editors:*

**GAETANO CREPALDI**

Department of Internal Medicine  
University of Padova  
Padova, Italy

**ANTONIO TIENGO**

Department of Internal Medicine  
University of Padova  
Padova, Italy

**GIULIANO ENZI**

Department of Internal Medicine  
University of Padova  
Padova, Italy



1990

EXCERPTA MEDICA, Amsterdam — New York — Oxford

© 1990 Elsevier Science Publishers B.V. (Biomedical Division)

All rights reserved. No part of this publication may be reproduced, stored in a retrieval system or transmitted in any form or by any means, electronic, mechanical, photocopying, recording or otherwise without the prior written permission of the publisher, Elsevier Science Publishers B.V., Biomedical Division, P.O. Box 1527, 1000 BM Amsterdam, The Netherlands.

No responsibility is assumed by the Publisher for any injury and/or damage to persons or property as a matter of products liability, negligence or otherwise, or from any use or operation of any methods, products, instructions or ideas contained in the material herein. Because of rapid advances in the medical sciences, the Publisher recommends that independent verification of diagnoses and drug dosages should be made.

Special regulations for readers in the USA - This publication has been registered with the Copyright Clearance Center Inc. (CCC), 27 Congress Street, Salem, MA 01970, USA. Information can be obtained from the CCC about conditions under which photocopies of parts of this publication may be made in the USA. All other copyright questions, including photocopying outside the USA, should be referred to the copyright owner, Elsevier Science Publishers B.V., unless otherwise specified.

International Congress Series No. 872  
ISBN 0 444 81151 6

*This book is printed on acid-free paper.*

*Published by:*  
Elsevier Science Publishers B.V.  
(Biomedical Division)  
P.O. Box 211  
1000 AE Amsterdam  
The Netherlands

*Sole distributors for the USA and Canada:*  
Elsevier Science Publishing Company Inc.  
655 Avenue of the Americas  
New York, NY 10010  
USA

## **PREFACE**

This volume contains the papers presented at the 5th European Symposium on Metabolism (Padova, 15-17 May 1989) by the invited speakers, and some selected oral presentations. These Symposia, held in Padova every four years, are a must for all scientists interested in lipoprotein, adipose tissue metabolism, and diabetes.

The current Symposium focused on genetics and the role of apoprotein on lipid metabolism, the disorders of lipoprotein metabolism in diabetes mellitus, and the enzymes involved in lipid metabolism. With respect to obesity, the papers deal with the development and differentiation of adipose tissue, the new trends in pathogenesis, and in the importance of different fat distribution. Furthermore, fuel substrate utilization and interaction in diabetes mellitus and the pathogenesis and treatment of diabetic nephropathy are discussed.

For the second time, the Morgagni Prize, endowed by Fidia Research Laboratories under the auspices of the Faculty of Medicine of the University of Padova, was awarded during the Symposium. Dr. Willy J. Malaisse, whose lecture opens this book, was the recipient of what is today one of the most important prizes offered to scientists working in the field of metabolism.

This volume contains some really up-to-date work on different, but at the same time related, aspects of metabolism, and we hope it will prove to be a valuable addition to International literature.

**GAETANO CREPALDI**

Chairman of  
the 5th European  
Symposium on Metabolism

# CONTENTS

## MORGAGNI LECTURE

- Physiology and pathology of the pancreatic  $\beta$ -cell glucose-sensor device  
*W.J. Malaisse* 3

## LIPOPROTEINS AND ATHEROSCLEROSIS

- Molecular genetics of coronary atherosclerosis: Variation at the LPL locus  
*J. Thorn, C. Chamberlain, J. Stocks, K. Oka and D.J. Galton* 25  
 Apo (a), A-I and B levels, lipoprotein subfractions and susceptibility to coronary artery disease (CAD)  
*A. Bishop, T.W. Young, R. Morgan, S.B. Matthews, D.G. Ephraim and A. Rees* 31  
 Effects of different fatty acids on lipid metabolism and cell membrane lipid composition  
*A. Pagnan and A. Bonanome* 35  
 Lipids and lipo-apoproteins in patients with chronic renal failure on hemodialytic therapy  
*M.R. Averna, C.M. Barbagallo, A. Galione, M. Labisi, G. Marino, G. Traina and A. Notarbartolo* 41

## LIPOPROTEIN AND APOPROTEIN METABOLISM

- Regulation of plasma high density lipoprotein concentration  
*M.-R. Taskinen, J. Kahri, V. Koivisto, T. Kuusi, K. Aalto-Setälä, H. Miettinen and K. Kontula* 47  
 Cholesterylester transfer reactions and plasma lipoprotein metabolism  
*A. Van Tol and J.E.M. Groener* 53  
 The esterified/free cholesterol ratio in LDL influences its ability to regulate intracellular cholesterologenesi  
*D. Owens, A. Johnson, P. Collins and G. Tomkin* 61  
 Apolipoprotein B mutants associated with familial hypobetalipoproteinemia  
*C. Gabelli, C. Bilato, G. Baggio, S. Martini, L. Previato, M. Albanese, Y.L. Marcel and G. Crepaldi* 65  
 The first mutations of the LDL receptor gene found in Italians  
*S. Bertolini, A. Daga, D.A. Coviello, E. Zucchetto, R. Balestreri, M. Ghisellini, N. Lelli, S. Fazio, M. Arca and S. Calandra* 73

|  |    |
|--|----|
| Apolipoprotein E polymorphism in northern Italy: Impact of allelic variation on lipid and lipoprotein levels in primary hyperlipoproteinemias and in healthy subjects<br><i>S. Martini, C. Gabelli, G. Baggio, G.M. Barbato, M.C. Corti, S. Pigozzo, A. Gasparotto, C. Bilato, M. Albanese and G. Crepaldi</i> | 79 |
| Plasma apolipoprotein B forms in non-mammalian species: Absence of apo B-48 in the chicken<br><i>P. Tarugi, L. Albertazzi, S. Nicolini and S. Calandra</i>   | 83 |

## NEW DEVELOPMENTS IN HYPOLIPIDEMIC THERAPY

|   |     |
|---|-----|
| Role of diet in the treatment of hyperlipidemias<br><i>M. Mancini and M. Parillo</i>                                      | 91  |
| Rationale for the choice of a hypolipidemic drug<br><i>J. Davignon</i>  | 99  |
| Management of hyperlipidemia<br><i>J. Shepherd, M.J. Caslake, J.J. Series and C.J. Packard</i>                            | 105 |
| Clinical trials with nicotinic acid in the prevention of atherosclerosis<br><i>G. Valerio, F. Romagnoni and R. Fellin</i> | 113 |
| Maximal therapy of familial hypercholesterolemia in CAD-patients<br><i>J. Thiery and D. Seidel</i>                        | 119 |

## HMG-CoA REDUCTASE INHIBITORS

|  |     |
|--|-----|
| Overview of the pharmacology and mechanism of action of HMG-CoA reductase inhibitors<br><i>C.R. Sirtori</i>  | 131 |
| Cholesterol metabolism during hypolipidemic treatment with inhibitors of cholesterol synthesis<br><i>T.A. Miettinen</i>  | 139 |
| Simvastatin in combination with other lipid lowering drugs (colestipol and fenofibrate): Effects on serum lipoproteins<br><i>P. Weisweiler</i>   | 145 |
| Effect of simvastatin on lipid and hormonal levels in patients with familial hypercholesterolemia<br><i>G.P. Balestrieri, A. Salvi, O. Di Stefano, S. Spandrio, M.C. Cotelli and R. Candrina</i> | 149 |

## DEVELOPMENT AND DIFFERENTIATION OF ADIPOSE TISSUE

|   |     |
|---|-----|
| Control of differentiation of adipocyte precursor cells (islet-derived cells) by mature adipocytes in cell culture<br><i>R. Carraro</i> | 155 |
|---|-----|

|   |     |
|---|-----|
| Differentiation of preadipocytes from different types of adipose tissues in vitro: A morphological study<br><i>S. Cinti, M. Cigolini and M. Morroni</i> | 161 |
| Differentiation of human adipocyte precursor cells of primary culture<br><i>H. Hauner</i>   | 167 |
| Steroid hormone effects on adipose tissue growth and metabolism<br><i>X. Xu, G. De Pergola and P. Björntorp</i>   | 173 |

## NEW TRENDS IN THE PATHOGENESIS OF OBESITY

|   |     |
|---|-----|
| Neuro-endocrinology of Type 2 diabetes in animal models<br><i>B. Jeanrenaud</i>   | 181 |
| Energy predictors of body weight gain<br><i>E. Ravussin and C. Bogardus</i>   | 189 |
| Food induced thermogenesis and the pathogenesis of human obesity<br><i>L. Scalfi, A. Coltorti and F. Contaldo</i>   | 197 |
| Determinants of energy substrate utilization: Low fat oxidation is a predictor of body weight gain<br><i>F. Zurlo and E. Ravussin</i>   | 205 |
| Insulin sensitivity in non insulin dependent diabetes mellitus and obesity with the minimal model approach. Importance of early insulin secretion in the carbohydrate intolerance<br><i>D. Arauxo, E. Camarero, M. Iglesias-Guerrero and J. Cabezas-Cerrato</i> | 211 |
| Sleep disturbances in obese infants<br><i>W. Burniat, E. Rebuffat, M.J. Mozin and A. Kahn</i>   | 215 |
| Chronic ethanol exposure and ethyl esters of fatty acids in adipose tissue of rats<br><i>G. De Pergola, C. Kielstrom, N. Conradi and P. Björntorp</i>   | 219 |
| Central GABAergic control of insulin secretion in experimental and human obesity<br><i>F. Squadrito, D. Cucinotta, F. Corica, V. Arcoraci, R. Sturniolo, A.P. Caputi and G. Squadrito</i>   | 223 |

## FAT DISTRIBUTION AND CLINICAL MANIFESTATION OF OBESITY

|  |     |
|--|-----|
| Adipose tissue distribution and development of diabetes mellitus<br><i>P. Björntorp</i>  | 229 |
| Steroid hormone effects on adipose tissue distribution and metabolism<br><i>M. Rebuffé-Scrive</i>  | 233 |
| Abdominal fat modifications induced by severe caloric restriction: Their relationships with plasma C-peptide and insulin levels<br><i>O. Bosello, M. Zamboni, F. Armellini, I. Zocca, L. Cominacini and L.A. Scuro</i> | 239 |

|   |     |
|---|-----|
| The obesity-hyperandrogenism association in premenopausal women<br><i>R. Pasquali, F. Casimirri and S. Cantobelli</i>   | 247 |
| Visceral fat accumulation in men: Associations with glucose intolerance and testosterone levels<br><i>J.C. Seidell, P. Björntorp, L. Sjöström, H. Kvist, R. Sannerstedt and M. Krotkiewski</i>  | 257 |
| Influence of body fat distribution on metabolic and haemodynamic parameters in young men<br><i>E. Bonora, M. Zenere, P. Branzi, M. Bagnani, L. Maggiulli, F. Tosi, D. Travia, V. Cacciatori, M. Quèrena, P. Moghetti and M. Muggeo</i>  | 261 |
| Coagulation and fibrinolysis factors in relation to body fat distribution and plasma insulin in a randomized sample of 38-year-old men<br><i>M. Cigolini, R. Schiavon, L. Zambelli, D. Benati, A. Turrini, P. Branzi, M. Balzanelli, P. Falcieri, A. Lechi and L.A. Scuro</i> | 265 |
| Hormonal, metabolic and anthropometric features of obese women with lowered sex hormone-binding globulin concentrations<br><i>R. Pasquali, F. Casimirri, L. Plate, M. Capelli, S. Cantobelli, N. Melchionda and L. Barbara</i>  | 269 |

## FUEL SUBSTRATE INTERACTIONS IN DIABETES MELLITUS

|  |     |
|--|-----|
| Changes in glucose metabolism and in insulin sensitivity during the suckling-weaning transition in the rat<br><i>C. Coupe, T. Issad, D. Perdureau, P. Ferre and J. Girard</i>  | 275 |
| Interplay of epinephrine and insulin in the regulation of fatty acid and glucose flux in humans<br><i>P.E. Cryer, A. Avogaro and D.M. Bier</i>   | 283 |
| Euglycemic Type I insulin dependent diabetic subjects have normal ketogenic epinephrine responsiveness<br><i>A. Avogaro, R. Nosadini, A. Doria, A. Valerio, L. Gnudi, M. Dorella, M. Miola, A. Tiengo, P.E. Cryer, G. Crepaldi and D.M. Bier</i> | 289 |
| Adrenaline opposes inhibition of cardiac PDH by lipid fuels in vivo<br><i>M.C. Sugden, A.W. Goode and T.J. French</i>  | 293 |
| Substrate production and utilization during insulin-induced hypoglycaemia<br><i>G.B. Bolli, P. De Feo, G. Perriello, E. Torlone, C. Fanelli, F. Santeusano and P. Brunetti</i>   | 297 |
| Sustained effects of glucagon on increased hepatic glucose production during counterregulation in man<br><i>P. De Feo, G. Perriello, E. Torlone, C. Fanelli, F. Santeusano, P. Brunetti and G.B. Bolli</i>                                       | 311 |
| Amino acid metabolism in skeletal muscle and in the splanchnic bed in diabetes: A mini-review of past and new data<br><i>P. Tessari, S. Inchiostro, G. Biolo, D. Bruttomesso and A. Tiengo</i>   | 317 |



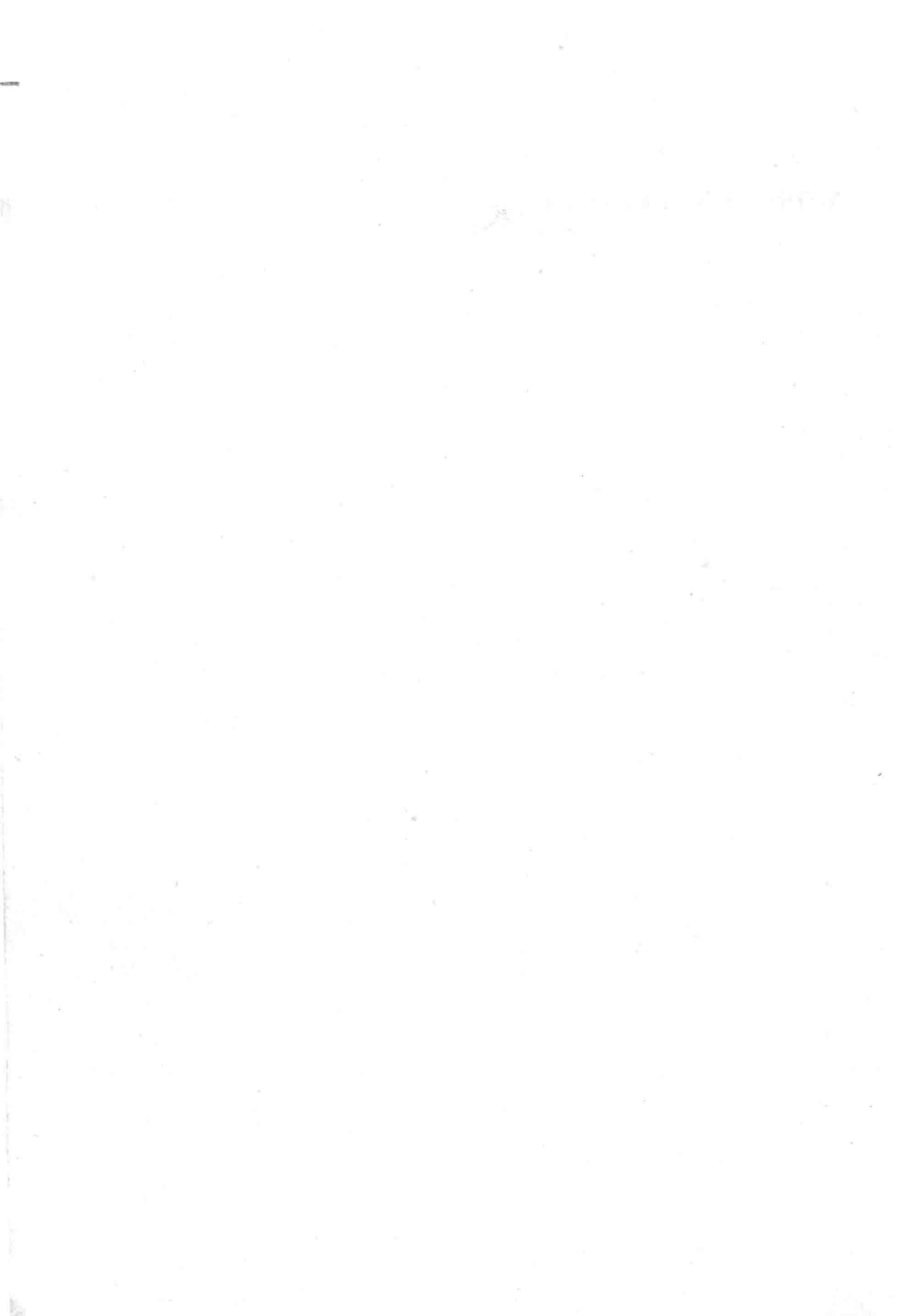
|  |     |
|--|-----|
| Glucose and amino acids interactions: 'in vitro' and 'in vivo' results<br><i>L. Luzi and G. Pozza</i>  | 325 |
| Fatty acid chain length affects leucine metabolism during parenteral infusions of triglycerides<br><i>M.W. Haymond, P. Tessari, B. Beaufrere, N. Rodriguez, J. Bailey and J.M. Miles</i> | 333 |
| Can NEFA levels modify the non-insulin and the insulin mediated forearm glucose uptake in normal man?<br><i>P.M. Piatti, L.D. Monti, M. Pacchioni, A.E. Pontiroli and G. Pozza</i>       | 339 |
| Insulin receptor structure investigated by antipeptide antibodies<br><i>A. Pessino, R. Gherzi, E. Manera, G. Damiani and R. Cordera</i>  | 343 |
| In vivo and in vitro insulin processing produces superimposable degradation products<br><i>L. Benzi, A.M. Ciccarone, P. Cecchetti, G. Di Cianni, L.C. Iozzi and R. Navalesi</i>          | 349 |
| Effects of insulin and diabetes mellitus on myocardial glucose and glycogen metabolism in vivo<br><i>E.J. Barrett, M.R. Laughlin and L.H. Young</i>                                      | 353 |
| Substrate interaction in diabetes of the obese<br><i>J.-P. Felber</i>  | 359 |
| Splanchnic exchange of glucose and gluconeogenic substrates in physiology and in Type II diabetes<br><i>L. Saccà, R. Napoli, P. Di Bonito, G. Albano, F. Cacciatore and B. Capaldo</i>   | 367 |
| Acute responses of protein, fat and carbohydrate utilization to nutrients in Type II diabetics<br><i>M.A. McNurlan, M. Zin, J. Broom and P.J. Garlick</i>                                | 373 |

## **PATHOGENESIS AND TREATMENT OF DIABETIC NEPHROPATHY**

|  |     |
|--|-----|
| Structure and clinical significance of pentosidine<br><i>D.R. Sell and V.M. Monnier</i>  | 379 |
| Mechanisms of glomerular permselectivity in diabetic nephropathy<br><i>R. Mangili</i>  | 387 |
| Genesis of diabetic renal and cardiovascular disease: A hypothesis<br><i>G. Viberti</i>  | 397 |
| Prostaglandins and kidney function in normal man<br><i>R. Nosadini, P. Fioretto, D. Sacerdoti, C. Giorato, A. Morocutti, A. Doria, A. Avogaro, R. Trevisan, C. De Donà, P. Faronato, F. Riva, F. Mollo, V. Donadon, M. Velussi, A. Mongillo and G. Viberti</i> | 409 |
| Metabolic control and development of late complications. A five year update on the Steno study<br><i>B. Feldt-Rasmussen, E.R. Mathiesen, T. Jensen and T. Deckert</i>  | 417 |

|  |     |
|--|-----|
| Novel hints in a pathogenetic therapy in diabetic kidney disease:<br>Pharmacological attempts  |     |
| <i>U. Di Mario, M. Sensi, G. Pugliese and F. Pugliese</i>  | 421 |
| Microalbuminuria and renal function in Type I diabetic patients:<br>Evolution and effect of glycemic control   |     |
| <i>A. Verrillo, A. De Teresa, C. Martino, G. Di Chiara, P. Cristiano,<br/>    F. Torello</i>   | 431 |
| Insulin influences the renin - angiotensin - aldosterone system in man   |     |
| <i>M. Trovati, P. Massucco, G. Anfossi, F. Cavalot, E. Mularoni,<br/>    L. Mattiello and G. Emanuelli</i>   | 435 |
| Evaluation of renal complication in insulin-dependent (Type I) diabetes  |     |
| <i>R. Lugari, P. Tagliavini, R. Sala, L. Bonomini, A.L. Barilli,<br/>    A. Strata, S. Caronna, P.G. Benedetti, R. Ronaia, R. Biagioli,<br/>    C. Dradi Maraldi, G. Madini, L. Boari, D.G. Pierfranceschi,<br/>    U. De Joannon and A. Gnudi</i> | 439 |
| High risk of cardiovascular disease in Type II diabetic patients with<br>clinical nephropathy  |     |
| <i>N. Pussariello, G. Paolisso, G. Marazzo, G. Pizza, S. Sgambato and<br/>    F. D'Onofrio</i>   | 443 |
| Tubular enzymuria in insulin-dependent (IDDM) and non-insulin<br>dependent (NIDDM) diabetic patients   |     |
| <i>O. Giampietro, G. Penno, L. Palmieri, S. Bertoli, G. Gregori,<br/>    L. Cruschelli, G. Ronca and R. Navalesi</i>   | 447 |
| Atrial natriuretic factor behaviour in hypertensive diabetic patients  |     |
| <i>G. De Mattia, C. Ferri, M.G. Tullo, O. Laurenti, C. Giarrizzo,<br/>    C. Ventura, A. Santucci and F. Balsano</i>   | 451 |
| Insulin stimulates Na,K-ATPase in human activated lymphocytes  |     |
| <i>C. Bozzo, M. Gorla, S. Marena and G. Pagano</i>   | 455 |
| Collisional spectroscopy in the identification of new advanced glycation<br>products on collagen samples of diabetic rats  |     |
| <i>C. Gerhardinger, A. Lapolla, E. Ghezzi, A. Sturaro, R. Seraglia,<br/>    A. Franchin, P. Traldi, G. Crepaldi and D. Fedele</i>  | 459 |
| Mitogenic activity of normal and diabetic serum on human resting<br>fibroblasts  |     |
| <i>C. Perrone and A. D'Alessandro</i>  | 463 |
| <b>INDEX OF AUTHORS</b>  | 466 |

## MORGAGNI LECTURE



## PHYSIOLOGY AND PATHOLOGY OF THE PANCREATIC B-CELL GLUCOSE-SENSOR DEVICE<sup>1</sup>

Willy J. MALAISSE

Laboratory of Experimental Medicine, Brussels Free University,  
Brussels, Belgium

### INTRODUCTION

Insulin secretion plays an essential role in the control of fuel homeostasis. The major theme of this contribution represents a complementary proposal, namely that fuel metabolism in the pancreatic B-cell plays an essential role in the control of insulin secretion. The capacity of D-glucose and other nutrient secretagogues to stimulate insulin release may indeed reflect their capacity to act as a fuel and, hence, to accelerate ATP generation rate in islet B-cells. This fuel concept was first formulated ten years ago (1, 2), after almost two decades of debate between the defenders of the glucoreceptor theory and those of the metabolic theory for insulin release. The essential findings in support of this fuel concept consist in the observation that the higher insulinotropic capacity of  $\alpha$ - than  $\beta$ -D-glucose (or D-mannose) coincides with a more efficient utilization of the  $\alpha$ - than  $\beta$ -anomers in pancreatic islets (3, 4) and the elucidation of the mode of action of non-metabolized or poorly metabolized nutrient secretagogues, especially the activation of glutamate dehydrogenase by the non-metabolized analogue of L-leucine, 2-amino[2,2,1]heptane-2-carboxylic acid (5, 6), and the role of 3-phenylpyruvate as a transamination partner in islet cells (7, 8).

When this fuel concept had been documented, it was considered that a further task, in order to progress in our understanding of the cytophysiology of insulin release, should aim at characterizing both the regulation of nutrient catabolism in normal B-cells and its perturbation in experimental models of B-cell dysfunction. The present account, which complements prior reports on the same theme (9, 10),

<sup>1</sup>The Morgagni Prize Lecture delivered on May 16, 1989, at the 5th European Symposium on Metabolism in Padova.

aims at reviewing recent acquisitions dealing with the physiology and pathology of the pancreatic B-cell glucose-sensor device (Table I).

TABLE I

## THE PANCREATIC B-CELL GLUCOSENSOR DEVICE

1. D-glucose transport
2. D-glucose phosphorylation
  - Hexokinase isoenzymes
  - Synarchistic regulation
  - Hexokinase binding
  - Glucokinase regulation
3. Hexose phosphates channelling
  - Anomeric specificity
  - Diastereomeric specificity
4. Phosphofructokinase activation
5. Mitochondrial oxidative events
  - Glycerol phosphate shuttle
  - Pyruvate decarboxylation
  - Acetyl coenzyme A oxidation
6. Anomeric specificity

## GLUCOSE TRANSPORT

The transport of D-glucose and other hexoses into the B-cell (but not into purified non-B islet cells) is sufficiently efficient to ensure a rapid equilibration of D-glucose concentrations across the plasma membrane (11, 12). This represents a first essential attribute of the B-cell glucose-sensor device. A site-specific defect in hexose transport was first identified in islet cells of the RINm5F line (13). In these tumoral cells, as distinct from normal B-cells, no rapid equilibrium of the extracellular and intracellular concentrations of D-glucose takes place especially at low temperatures or high hexose concentrations (14-16). Whether a comparable defect may be found in the B-cell of animals prone to become diabetic or affected by this disease remains a matter of debate (17).

#### GLUCOSE PHOSPHORYLATION

Although the B-cell is equipped with both a low-K<sub>m</sub> hexokinase and high-K<sub>m</sub> glucokinase, the phosphorylation of D-glucose under physiological conditions occurs mainly at the intervention of the latter enzyme, hexokinase being largely inhibited in intact cells by glucose 6-phosphate and, to a lesser extent, by glucose 1,6-bisphosphate (18).

The rate of D-glucose phosphorylation is not solely dependent, however, on the concentration of the hexose inside the B-cell.

First, it depends on the hexokinase isoenzyme pattern, which may be altered by environmental factors (e.g. in fasting) or in pathological situations (19, 20).

Second, ATP participates in a synarchistic and sequential-type regulation of glucose phosphorylation in islet cells (21).

Third, in islets, a major fraction of hexokinase isoenzymes is bound to mitochondria (22, 23). The binding of hexokinase is inhibited by D-glucose 6-phosphate and enhanced by spermidine. This ambiguity of hexokinase may play a propitious role in the glucose-sensing function of the B-cell, and this for several reasons. Bound hexokinase is indeed more resistant than the cytosolic enzyme to inhibition by D-glucose 6-phosphate (22). The mitochondrial binding of hexokinase also provides a direct pathway for the coupling of hexose phosphorylation to mitochondrial respiration (24). Most importantly, mitochondria-bound hexokinase uses mitochondrial rather than cytosolic ATP as a substrate for D-glucose phosphorylation. This was documented in isolated islet mitochondria incubated in the presence of D-[U-<sup>14</sup>C]glucose and [ $\gamma$ -<sup>32</sup>P]ATP (Fig. 1). In the absence of any other agent, only 40% of the rate of D-glucose phosphorylation is accounted for by the contribution of exogenous ATP, the major fraction of the phosphorylation process being supported by endogenous ATP. The fractional contribution of exogenous ATP is further decreased in the presence of ADP and succinate and, inversely, increased to close-to-unity in the presence of metabolic poisons including inhibitors of either adenine nucleotide translocation or adenylate kinase activity. This novel experimental approach unambiguously documents that

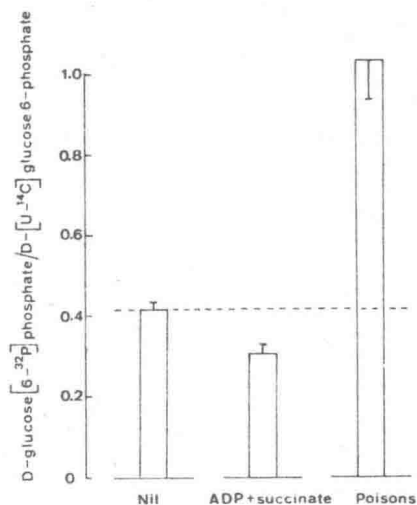


Fig. 1. Phosphorylation of D-[U-<sup>14</sup>C]glucose (1.0 mM) by islet mitochondria exposed to [ $\gamma$ -<sup>32</sup>P]ATP (0.4 mM) in the absence (Nil) or presence of either ADP (0.2-2.0 mM) and succinate 10.0 mM or KCN (1.0 mM), rotenone (10  $\mu$ M), carboxyatractyloside (12  $\mu$ M) and P<sup>1</sup>,P<sup>5</sup>-di-(adenosine-5')-pentaphosphate (10  $\mu$ M). The results refer to the fractional contribution of exogenous ATP to the total rate of hexose phosphorylation

mitochondria-bound hexokinase uses preferentially mitochondrially formed ATP as a substrate for D-glucose phosphorylation (25). This may help to maintain a high concentration of cytosolic ATP. At this point, it seems appropriate to recall that cytosolic ATP is currently considered as the key factor coupling the metabolism of nutrients to more distal cationic events in the secretory sequence (26).

Last, the activity of glucokinase may be modulated by a cytosolic regulatory protein mediating the antagonistic and competitive effects of D-fructose 1-phosphate and D-fructose 6-phosphate, as recently reported in hepatocytes (27, 28). The same mechanism may be operative in islet cells, provided that the latter are equipped with fructokinase in order to catalyze the formation of D-fructose 1-phosphate (Fig. 2). In prior reports, it was proposed that the phosphorylation of D-fructose in islet cells is catalyzed mainly by hexokinase



rather than fructokinase (29, 30). However, recent experiments indicate that fructokinase is nevertheless present in islet cells (31). In these experiments, islet homogenates were heated for 5 min at 70°C in order to abolish their capacity to catalyze the phosphorylation of D-glucose. A residual rate of D-fructose phosphorylation was still

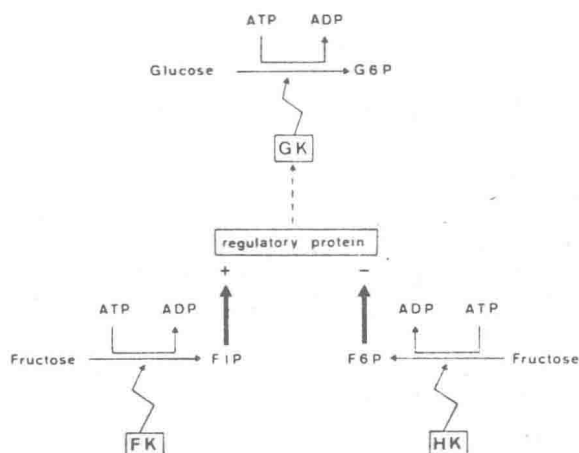


Fig. 2. Schematic view for the role of a regulatory protein mediating the antagonistic effects of D-fructose 1-phosphate (F1P) and D-fructose 6-phosphate (F6P), generated respectively in the fructokinase (FK) and hexokinase (HK) reaction, upon glucokinase (GK) activity.

observed, however, in the heated homogenates (Fig. 3, left panel). Moreover, the phosphorylation of D-fructose by the heated homogenate was strictly resistant to the presence of either D-glucose or D-glucose 6-phosphate and was abolished in the absence of  $K^+$ , all features characteristic of the participation of fructokinase (Fig. 3, right panel). Further work made it possible to document the presence in pancreatic islet of a regulatory protein indeed conferring to glucokinase the property of being antagonistically affected by D-fructose 1-phosphate and D-fructose 6-phosphate (32).